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Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

JUSTIN L. POWERS, EDITOR, WASHINGTON, D. C.

VOLUME XXXVII — *NOVEMBER, 1948* — NUMBER 11 — CONSECUTIVE NO. 22

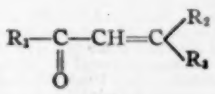
The Antibacterial Activity of Some Beta-Aroyl Acrylic Acids, Esters and Amides*

By BETTY JANE CRAMER[1], WILLIAM SCHROEDER[2], WILLIAM J. MORAN[3], CYRIL H. NIELD[4], MARGUERITE EDWARDS[5], CHARLES I. JAROWSKI[6], and BRUNO PUETZER†

Methods of preparing a series of ring-substituted beta-aroyl acrylic acids, esters, and amides are described. The bacteriological properties of these compounds are reported. Compounds containing alkyl groups in the ortho positions relative to the unsaturated side chain were found to be most effective.

In 1945 Geiger and Conn (1) postulated a mechanism for the antibiotic action of clavacin and penicillic acid, which is dependent upon their reaction with the sulfhydryl groups in bacterial enzyme systems or with sulfhydryl-containing metabolites essential to bacteria. This theory originated from their observation that both of these compounds contain a common structural feature; namely, the —CH=C—C=O group. In support of their claims they demonstrated that clavacin and penicillic acid are inactivated by an excess of a sulfhydryl compound and further that each of these compounds, when present in excess, abolishes the nitroprusside reaction of cysteine or thioglycollate. A similar inactivation has also been reported by other investigators (2, 3).

Based on chemical studies on the addition reactions of α,β-unsaturated ketones, Geiger and Conn (1) concluded that a ketone will undergo addition reactions most readily when R_1 of Formula I is an aromatic radical and R_2 and R_3 are hydrogens.



(I)

In conformance with the above postulate they found that acrylophenone (R_1 = phenyl; R_2 and R_3 = H) was actively bacteriostatic and that it imitated clavacin since its activity was eliminated by the presence of cysteine or thioglycollate.

In the course of an investigation to prepare benzenoid homologs of clavacin it was found that treatment of 2-carbethoxy-γ-chromanone (II) with sodium hydroxide gave β-(*o*-hydroxyben-

* Received April 1, 1948, from the Research Laboratories, Vick Chemical Company, Flushing, N. Y.

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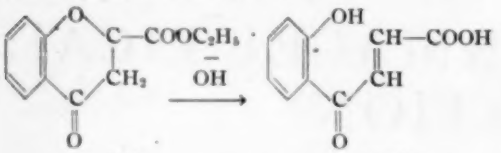
† The authors wish to thank Dr. M. K. McPhail for the toxicity data. For the analytical values we are indebted to Mr. Glenn B. Hess and Miss Celia Durham. To Dr. Thomas C. Grubb we express our gratitude for his many suggestions and consistent assistance.

zoyl)-acrylic acid (III). This reaction is analogous to the conversion of the flavanone, hesperitin, to 2,4,6-trimethoxyphenyl-3,4-dimethoxystyryl ketone by means of dimethyl sulfate and potassium hydroxide (4).



(II) (III)

A mechanism for the opening of the pyran ring has been suggested in a recent paper (5).

In compound (III) can be seen the structure which Geiger and Conn (1) suggested for effective antibacterial action. From Table I it is evident that β-(*o*-hydroxybenzoyl)-acrylic acid exhibited a marked bacteriostatic activity against both Gram-positive and Gram-negative organisms.

Since the β-benzoyl acrylic acids as a class would represent an improvement over the acylophenone structure in so far as stability, solubility, and ease of preparation are concerned, a series of β-aroyl acrylic acids and related compounds was prepared in an effort to find compounds with even greater antibacterial activity. These compounds are listed in Tables II, III, IV, and V.

ene, tetrachlorethane or an excess of the benzenoid compound). Aluminum chloride (anhydrous) was used in 0.2 mole excess after molar quantities had been calculated to react with the oxygen of phenols, ethers, and carbonyl groups. During the slow addition of aluminum chloride, the mixture was kept at various temperatures ranging from 10° to 25° depending on the vigor of the reaction. Mechanical stirring was continued at room temperature or up to 100° from one to three hours. When the evolution of HCl subsided the reaction mixture was decomposed with dilute HCl. In some cases the acid precipitated from the solvent or from the aqueous acid solution after the removal of the organic solvent by steam distillation. The product was purified by reprecipitation from a charcoaled sodium bicarbonate solution and recrystallization from benzene, alcohol, dilute alcohol, or glacial acetic acid.

Procedure II: Preparation of Esters.—The esters were prepared from the acids by refluxing with alcoholic compounds containing 2% anhydrous HCl as a catalyst.

Procedure III: Preparation of Amides and Anilides.—The β-aroyl acrylic acid chloride, prepared from the acid (1 mole) and phosphorous pentachloride (1 mole), was treated at 0–15° with the amine (1 mole) and pyridine (2 moles). The reaction was carried out in anhydrous ether or dioxane depending upon the solubility of the amine. When ether was used as a solvent, the reaction mixture was extracted with dilute hydrochloric acid and saturated sodium bicarbonate solution, dried, and then the ether removed. The residue was treated with a mixture of "Skellysolve A" and ether; the product was fil-

TABLE I.—THE BACTERIOSTATIC ACTION OF β-(*o*-HYDROXYBENZOYL)-ACRYLIC ACID

Organism	Serial Dilution Test Method[d] 1 × 10⁴	2 × 10⁴	4 × 10⁴	8 × 10⁴	16 × 10⁴	32 × 10⁴
Staphylococcus aureus	−[a]	−	−	−	+[b]	+
Staphylococcus albus	−	−	−	−	+	+
Bacillus subtilis	−	−	−	−	+	+
Escherichia coli	+[c]	+	+	+	+	+
Eberthella typhosa (Hopkins)	−	−	−	−	+	+
Bacillus prodigiosum	±	±	±	+	+	+
Salmonella paratyphi A	−	−	−	+	+	+
Salmonella paratyphi B	−	−	−	±	+	+
Shigella paradysenteriae (Flexner)	−	−	−	+	+	+
Shigella sonne	...	+	+	±	+	+
Trichophyton mentagrophytes (*spore suspension*)	+	+	+	+	...	...

[a] Complete inhibition.
[b] No inhibition.
[c] Partial inhibition.
[d] Method of testing (Serial Dilution Test).—*Medium:* Neopeptone-dextrose broth for fungi, F D A nutrient broth for bacteria. *Procedure:* Make 1: 1,000,000 dilution of culture in broth. Pipette 1 ml. inoculated broth into sterile 10 × 70-mm. tubes. To first tube add 1 ml. solution to be tested, mix well with pipette and remove 1 ml. to second tube. Repeat until the required number of dilutions is made. Leave last tube for control of culture growth. Read after incubating for exactly twenty-four hours at 37°. Incubate fungi cultures for ten days at 25°. Compounds difficultly soluble in water were submitted for testing in dilute alcohol. Only those dilutions which contained less than 3% by volume of alcohol were considered, for higher concentrations were found to be inhibitory to the growth of *S. aureus* and *E. typhosa*.

EXPERIMENTAL

Preparation of Compounds

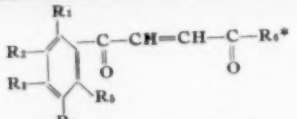
Procedure I: Preparation of β-Aroyl Acrylic Acids.—The general method for the acylation of aromatic compounds by maleic anhydride and aluminum chloride consisted of mixing the aromatic compound and the anhydride (mole for mole) in an appropriate solvent (carbon disulfide, nitrobenz-

tered off and recrystallized from either dilute acetic acid, benzene, or nitromethane.

When dioxane was used as a solvent, the reaction mixture was drowned in dilute hydrochloric acid and the precipitated solid filtered off. The solid was ground in a mortar with a saturated sodium bicarbonate solution, filtered, washed with water, dried, and recrystallized.

Procedure IV: Addition of Active Hydrogen Compounds.—These reactions were carried out by

TABLE II.—THE BACTERIOSTATIC ACTION OF SOME β-BENZOYL ACRYLIC ACIDS, ESTERS, AND AMIDES



No.	R_1	R_2	R_3	R_4	R_5	R_6	M. p. (Unc.), °C.	B. p. (Unc.), °C.	Method of Prep.	Highest Dilution Causing Inhibition of Growth† *S. aureus***	Highest Dilution Causing Inhibition of Growth† *E. typhosa***
1	H	H	H	H	H	OH	96[a]	.....	I	1:40,000	.....[b]
2	H	H	Me	H	H	OH	136– 7[c]	.....	I	1:40,000	.....
3	Me	H	Me	H	Me	OH	141[d]	.....	I	1:200,000	.....
4	Et	H	Et	H	Et	OH[e]	100– 1.5	.....	I	1:400,000	1:100,000
5	H	H	Cl	H	H	OH[f]	159–61	.....	I	1:40,000	1:80,000
6	Cl	H	Cl	H	H	OH[g]	192– 4	.....	I	1:40,000	1:20,000
7	H	H	Cl	Cl	H	OH[h]	143	.....	I	1:80,000	1:20,000
8	OH	H	H	H	H	OH[i]	184	.....	..	1:80,000	1:80,000
9	H	H	OMe	H	H	OH	134[j]	.....	I	1:40,000	.....
10	H	H	HCONH	H	H	OH[k]	244– 5	.....	I	.....	.....
11	H	H	CH_2CONH	H	H	OH[l]	239	.....	I	.....	.....
12	H	NO_2	H	H	H	OH[m]	201– 2	.....	..	.....	.....
13	OH	H	H	Cl	H	OH	191	.....	I	1:40,000	1:40,000
14	OH	Br	H	Br	H	OH[n]	193– 5	.....	I	.....	.....
15	Me	H	H	CH_3CONH	H	OH[o]	184	.....	I	.....	.....
16	Me	CH_3CONH	H	H	H	OH	210–13	.....	I	.....	.....
17	OH	CH_3CONH	H	H	H	OH[p]	205– 6	.....	I	.....	.....
18	OH	H	H	CH_3CONH	H	OH	197– 8	.....	I	.....	.....
19	Me	H	OH	$(CH_3)_3CH$	H	OH	160	.....	I	.....	.....
20	CH_3COO	H	H	Me	H	OH	134	.....	I	1:80,000	1:20,000

* The open structure has been assigned to all these compounds on the basis of a polarographic study carried out on compounds 1, 3, 26, 30, 35, and 42 (8). These data showed half-wave potential values indicating reduction of the ketone group, thus eliminating the possibility that at least these six compounds did not exist in the cyclic form. This is in conformance with the findings of others (9).

† For the method of testing see Table I.

** *Staphylococcus aureus* No. 209 and *Eberthella typhosa* (Hopkins), 24-hr. cultures.

[a] M. p. reported, 96–7° (10). The activity has been reported by H. Rinderknecht, *et al.*, as 1:25,000 (11).

[b] No activity in lowest dilution tested.

[c] M. p. reported, 138° (12).

[d] M. p. reported, 140.5° (13).

[e] Calcd. for $C_{13}H_{16}O_3$: N. E., 260. Found: N. E., 257.

[f] M. p. reported, 157–8°. The activity has been reported as 1:50,000 by Rinderknecht, *et al.* (11).

[g] M. p. reported, 191–3°. The activity reported was 1:80,000 by Rinderknecht, *et al.* (11).

[h] Calcd. for $C_{10}H_6O_3Cl_2$: C, 49.01; H, 2.47. Found: C, 49.36; H, 2.70. Oxidation with alkaline permanganate gave 3,4-dichlorbenzoic acid, m. p. 202–3°; m. p. reported, 201–2° (14).

[i] Calcd. for $C_{10}H_8O_4$: C, 62.5; H, 4.19. Found: C, 62.12; H, 4.35.

[j] M. p. reported, 134° (15).

[k] Calcd. for $C_{11}H_9O_4N$: C, 60.27; H, 4.14. Found: C, 60.24; H, 4.58.

[l] Calcd. for $C_{11}H_{11}O_4N$: C, 61.7; H, 4.8; N, 6.01; N. E., 233. Found: C, 61.73; H, 4.94; N, 6.02; N. E., 234. Oxidation with alkaline permanganate gave *p*-acetylamino benzoic acid, m. p. 251°; m. p. reported, 250° (16).

[m] Prepared by nitration of β-benzoyl acrylic acid. Oxidation with alkaline permanganate gave *m*-nitrobenzoic acid, m. p. 141°; m. p. reported, 140–1° (17).

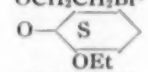
[n] Calcd. for $C_{10}H_8O_4Br_2$: C, 34.3; H, 1.75. Found: C, 34.2; H, 1.75.

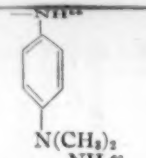
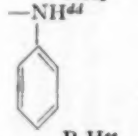
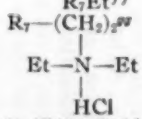
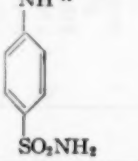
[o] Calcd. for $C_{11}H_{12}O_4N$: C, 63.16; H, 5.26; N. E., 247. Found: C, 63.34; H, 5.68; N. E., 247.

[p] Calcd. for $C_{12}H_{13}O_4N$: N, 5.63; N. E., 249. Found: N, 5.55; N. E., 123. The phenolic group was titrated also.

(*Cont'd on p. 442*)

TABLE II.—THE BACTERIOSTATIC ACTION OF SOME β-BENZOYL ACRYLIC ACIDS, ESTERS, AND AMIDES—(*Cont'd from p. 441*)

No.	R_1	R_2	R_3	R_4	R_5	R_6	M. p. (Unc.), °C.	B. p. (Unc.) °C.	Method of Prep.	Highest Dilution Causing Inhibition of Growth† *S. aureus***	Highest Dilution Causing Inhibition of Growth† *E. typhosa***
21	OH	Me	H	Me	H	OH[q]	195–96	.....	I	.....	.....
22	OH	H	Me	H	H	OH	170– 2	.....	I	1:80,000	.....
23	H	Me	OH	H	H	OH	168–70	.....	I	1:20,000	.....
24	OH	Me	H	H	H	OH[r]	176– 7	.....	I	1:300,000	1:20,000
25	OH	H	H	Me	H	OH[s]	174– 6	.....	I	1:80,000	1:20,000
26	H	Me	Cl	H	H	OH[t]	126– 8	.....	I	1:160,000	1:40,000
27	OH	H	Me	Cl	H	OH[u]	206– 9	.....	I	1:160,000	1:40,000
						Esters					
28	H	H	H	H	H	OMe	....	138–40 (3)[v]	II	1:200,000	.....
29	H	H	H	H	H	OEt	54[w]	.....	..	1:400,000	1:200,000
30	H	H	H	H	H	OEt	....	120–25 (2)[x]	II	1:400,000	1:200,000
31	H	H	H	H	H	$OCH_2CH{=}CH_2$	....	146– 8 (2)	II	1:400,000	.....
32	H	H	H	H	H	OCH_2CH_2Cl[y]	....	163– 5 (3)	II	1:800,000	.....
33	H	H	H	H	H	OCH_2CH_2Br[z]	54	168–70 (1)	II	1:400,000	.....
34	H	H	H	H	H	O– 	....	178–80 (1)	II	1:800,000	1:100,000
35	Me	H	Me	H	Me	OEt	....	155– 6 (3)	II	1:400,000	.....
36	Me	H	Me	H	Me	OCH_2CH_2Cl	....	168–70 (1.5)	II	1:800,000	.....
37	Me	H	Me	H	Me	OBz	....	225 (0.8)	II	.....	.....
38	Me	H	Me	H	Me	$O{-}CH_2{-}CH_2{-}N(Et)(Et)\cdot HCl$	84–90	.....	II	.....	.....
39	Cl	H	H	H	H	OEt[aa]	59–60	156– 8 (0.7)	II	1:400,000	1:200,000
40	Cl	H	Cl	H	H	OEt	....	146 (0.5)	II	1:800,000	1:100,000
41	H	H	CH_2CONH	H	H	OEt	118–21	.....	II	1:400,000	1:100,000
42	H	Me	Cl	H	H	OEt	....	145–50 (0.3)	II	1:600,000	1:200,000
43	H	Me	Cl	H	H	$O{-}CH_2{-}CH_2{-}N(Et)(Et)\cdot HCl$	143–5	.....	II	.....	.....
44	OH	Me	H	H	H	OMe	118–20	.....	II	.....	1:100,000
45	OH	Me	H	H	H	OEt	82– 5	.....	II	.....	1:100,000
46	OH	H	H	Me	H	OMe	100	.....	II	.....	.....
47	OH	H	H	Me	H	OEt	60	.....	II	1:100,000	1:100,000
48	OH	H	Me	H	H	OMe	110–14	.....	II	.....	1:100,000
						Amides					
49	H	H	H	H	H	$R_7 = -N(H)-C_6H_3(C(=O)O-)-C(=O)-$	115	.....	III	1:200,000	1:200,000
50	H	H	H	H	H	—NH[ab]	197– 8	.....	III	1:200,000	.....

49	H	H	H	H	H		115	.....	III	1:200,000	1:200,000
50	H	H	H	H	H		197–8	.....	III	1:200,000	.....
51	Me	H	Me	H	Me	NH_2[ee]	153–4	.....	III	1:400,000	.....
52	Me	H	Me	H	Me		201–2	.....	III	1:800,000	.....
53	Me	H	Me	H	Me	R_7H[ee]	257–9	.....	III	1:800,000	.....
54	Me	H	Me	H	Me	R_7Et[ff]	154–5	.....	III	1:2,000,000	.....[ii]
55	Me	H	Me	H	Me		142–5	.....	III	1:200,000	.....
56	Me	H	Me	H	Me	$R_7(CH_2)_2CH_3$[hh]	113–6	.....	III	1:800,000	.....
57	Me	H	Me	H	Me	$R_7(CH_2)_3CH_3$	127–8	.....	III	1:800,000	.....
58	Me	H	Me	H	Me		258–9	.....	III	1:1,600,000	.....

[q] Calcd. for $C_{12}H_{12}O_4$: C, 65.45; H, 5.45. Found: C, 65.23; H, 5.66.
[r] Calcd. for $C_{11}H_{10}O_4$: C, 64.06; H, 4.88; N. E., 206. Found: C, 63.92; H, 5.10; N. E., 210.
[s] Calcd. for $C_{11}H_{10}O_4$: C, 64.06; H, 4.88. Found: C, 63.88; H, 4.88.
[t] Calcd. for $C_{11}H_9O_3Cl$: C, 58.8; H, 4.03. Found: C, 58.82; H, 4.11. Oxidation with alkaline permanganate gave 4-chloro-isophthalic acid, m. p. 294°; m. p. reported, 294.5° (18).
[u] Calcd. for $C_{11}H_9O_4Cl$: N. E., 240. Found: N. E., 245.
[v] B. p. reported, 185° (16 mm.) (19).
[w] M. p. reported, 54° (20).
[x] B. p. reported, 184–5° (25 mm.) (20).
[y] Calcd. for $C_{12}H_{11}O_3Cl$: Cl, 14.7. Found: Cl, 14.82.
[z] Calcd. for $C_{12}H_{11}O_3Br$: C, 50.88; H, 3.89. Found: C, 50.67; H, 4.2.
[aa] Calcd. for $C_{12}H_{11}O_3Cl$: Cl, 14.88. Found: Cl, 14.48.
[bb] Calcd. for $C_{18}H_{18}O_2N_2$: C, 73.44; H, 6.16. Found: C, 73.43; H, 6.6.
[cc] Calcd. for $C_{13}H_{13}O_2N$: C, 71.86; H, 6.95. Found: C, 72.07; H, 7.33.
[dd] Calcd. for $C_{13}H_{13}O_2N$: N, 4.77. Found: N, 4.73.
[ee] Calcd. for $C_{20}H_{19}O_4N$: C, 71.2; H, 5.6. Found: C, 70.88; H, 5.14.
[ff] Calcd. for $C_{23}H_{23}O_4N$: C, 72.3; H, 6.34. Found: C, 72.06; H, 6.03.
[gg] Calcd. for $C_{26}H_{29}O_4N_2Cl \cdot HOH$: C, 65.4; H, 7.2. Found: C, 65.52; H, 7.11.
[hh] Calcd. for $C_{23}H_{23}O_4N$: C, 72.79; H, 6.64. Found: C, 72.71; H, 6.84.
[ii] Calcd. for $C_{19}H_{20}O_4N_2S$: C, 60.83; H, 5.5. Found: C, 61.26; H, 5.4.
[jj] Raistrick Test (modified) for testing compounds which show marked activity in the screen test was used as follows:
Media.—F. D. A. Nutrient broth.
Organism.—*S. aureus* 209 and *E. typhi* (Hopkins, also others as requested).
Procedure.—Pipette 4 ml. broth into sterile 125 × 25-mm. tubes. Make serial dilutions of the solution to be tested in sterile 22 × 150-mm. test tubes with sterile distilled water. To each tube of broth add 1 ml. of the dilution. *Example:* First dilution 1:1000, after added to broth 1:5000. Second dilution 1:2000, after added to broth 1:10,000, etc.
Control.—1 ml. sterile distilled water added to 4 ml. broth.
Culture.—Dilute each culture (if good growth) 1:1000. With 0.2-ml. pipette add 0.01 ml. of diluted culture to each tube and controls. Incubate twenty-four hrs. at 37°.

TABLE III.—THE BACTERIOSTATIC ACTION OF SOME β-AROYL ACRYLIC ACIDS

No.	Compound	M. p. (Unc.), ° C.	Method of Prep.	Highest Dilution Causing Inhibition of Growth	
				S. aureus	*E. typhosa*
59		152	I	1:40,000	1:40,000
60		222	I	1:20,000	....
61		197–200	I	1:100,000	....

[a] Calcd. for $C_{14}H_{10}O_5S$: C, 52.7; H, 3.32; N. E., 182. Found: C, 52.62; H, 3.40; N. E. 180.
[b] Calcd. for $C_{19}H_{12}O_7$: N. E., 182. Found: N. E., 204.
[c] Calcd. for $C_{19}H_{17}O_4N$: N. E., 181. Found: N. E., 178.

mixing the β-aroyl acrylic acids or esters with an equimolar quantity of the HA compound (HA = compound containing active hydrogen) in alcohol. In some instances it was advantageous to react the sodium salts of the acids with the HA constituent in water. The adducts usually crystallized out on standing at room temperature. Where salts were used, acidification caused the separation of the addition compound. The products were purified by recrystallization from ethanol, dilute ethanol, or benzene.

Preparation of 2-Carbethoxy-γ-Chromanone.—One-tenth mole (21.8 Gm.) of 2-carbethoxy-γ-chromanone (6) was dissolved in 100 ml. of 95% ethanol and hydrogenated in the presence of one teaspoonful of Raney nickel. The pressure dropped from 50 to 40.3 lb. in twenty minutes (theoretical drop = 9.5 lb.). The catalyst was filtered off and the 2-carbethoxy-γ-chromanone was purified by distillation. Nineteen grams (86% yield) of a liquid was obtained; b. p. 3 = 153–4°.

Anal.—Calcd. for $C_{12}H_{12}O_4$: C, 65.5; H, 5.45. Found: C, 65.43; H, 5.58 (7).

Preparation of β-(*o*-Hydroxybenzoyl)-Acrylic Acid.—The above chromanone ester (19 Gm.) was dissolved in 40 ml. of 95% ethanol, and treated with 45 ml. of 20% sodium hydroxide at 50° for one minute. The reaction mixture was cooled and acidified immediately. The crude acid was purified by recrystallization from 50% ethanol; m. p. 184°, yield 8.2 Gm. (42%).

Anal.—Calcd. for $C_{10}H_8O_4$: C, 62.5; H, 4.19. Found: C, 62.12; H, 4.35.

Preparation of α-Ethoxy-β-(*p*-Chlorobenzoyl)-Ethyl Propionate.—This compound (No. 73) was a by-product in the esterification of β-(*p*-chlorobenzoyl)-acrylic acid by Procedure II. Boiling range: 156–158° (0.7 mm.).

Anal.—Calcd. for $C_{14}H_{17}O_4Cl$: C, 59.0; H, 5.96; Cl, 12.6. Found: C, 59.28; H, 5.81; Cl, 12.68.

Preparation of the Mercuric Acetate Adduct (No. 69).—Sixteen grams of β-benzoyl acrylic acid and 32 Gm. of mercuric acetate were dissolved in 150 ml. of methanol, heated on the steam bath for two hours and then cooled. The product was recrystallized from dilute dioxane; m. p. 168° (dec.), yield 16 Gm. (33%).

Anal.—Calcd. for $C_{14}H_{14}O_7Hg$; Hg, 40.54. Found: Hg, 40.61.

Effect of Cysteine Hydrochloride and *p*-Amino Benzoic Acid on Antibacterial Activity.—It was of interest to determine if excesses of cysteine hydrochloride or *p*-amino benzoic acid could nullify the antibacterial potency of these compounds. From Table VI it can be concluded that these substances do not alter the inhibitory power of β-(*o*-hydroxybenzoyl)-acrylic acid.

Effect of Serum on Activity.—As is the case with a host of other germicidal agents, our compounds lose activity in the presence of serum. This is evident from the results in Table VII.

The Antibacterial Activity of the *p*-Carbethoxy Anilide of β-Mesitoyl Acrylic Acid against Several Organisms.—Table VIII shows the inhibitory potency of one of the most active compounds against eight organisms. The test was carried out by the modified Raistrick method (see under Table II).

Toxicity.—The toxicity of the *p*-carbethoxy anilide of β-mesitoyl acrylic acid (No. 54) was deter-

TABLE IV.—THE BACTERIOSTATIC ACTION OF SOME β-BENZOYL PROPIONIC ACID DERIVATIVES

No.	Compound	M. p. (Unc.), ° C.	B. p. (Unc.), °C.	Method of Prep.	Highest Dilution Causing Inhibition of Growth	
					S. aureus	*E. typhosa*
62		114[a]	.....	I	.....[c]	.....
63		..	120 (0.8)[b]	II	.....	.....
64		148–52[a]	.....	IV	.....	.....
65		199–202	.....	IV	.....	.....
66		156–60	.....	IV	.....	1:80,000
67		202	.....	IV	.....	.....
68		171	.....	IV	.....	.....
69		168	.....	..	1:640,000	1:640,000
70		93–5	.....	IV	.....	.....

(Cont'd on p. 446)

TABLE IV.—THE BACTERIOSTATIC ACTION OF SOME β-BENZOYL PROPIONIC ACID DERIVATIVES.—(*Cont'd from p. 445*)

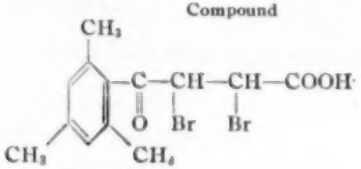
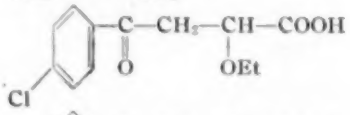
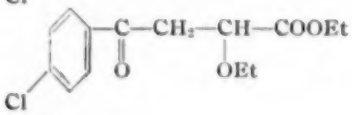
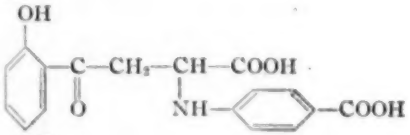
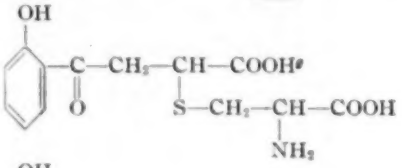
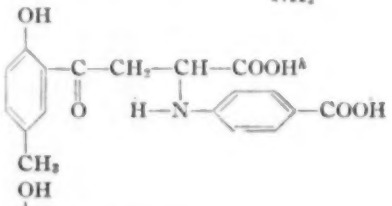
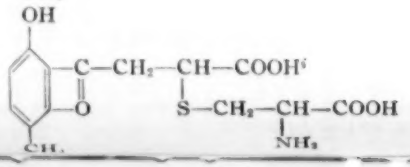
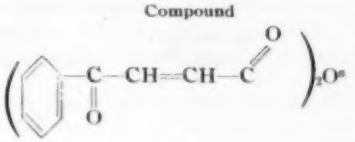
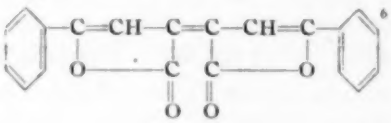
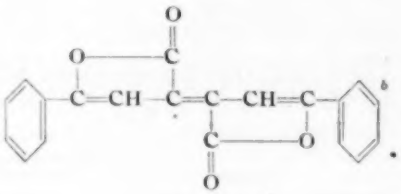
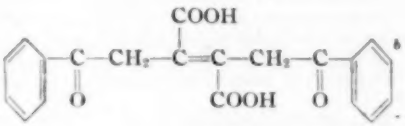
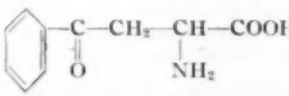
No.	Compound	N. p. (Unc.), °C	B. p. (Unc.), °C.	Method of Prep.	Highest Dilution Causing Inhibition of Growth *S. aureus*	Highest Dilution Causing Inhibition of Growth *E. typhosa*
71		167–9	.....	..	.....	.....
72		140	.....	..	.....	.....
73		..	156–8 (0.7)	II	.....	.....
74		198	.....	IV	.....	.....
75		169–70	.....	IV	.....	.....
76		190	.....	IV	.....	.....
77		165	.....	IV	.....	.....

TABLE V.—THE BACTERIOSTATIC ACTION OF SOME MISCELLANEOUS COMPOUNDS

No.	Compound	M. P. (Unc)° C.	Highest Dilution Causing Inhibition of Growth	
			S. aureus	*E. typhosa*
78		112–3	1:200,000	1:200,000
79	 [b]	...	.....*	1:476,000
80	 [b]	...	.....	1:408,000
81	 [b]	...	.....	.....
82	 [c]	...	.....	.....

* No activity in lowest dilution tested.
[a] Calcd. for $C_{10}H_{10}O_3$: C, 71.8; H, 4.2. Found: C, 72.12; H, 4.54. Structure proved by conversion to the theoretical amount of β-benzoyl acrylic acid.
[b] Prepared according to the method of Bogert and Ritter (24).
[c] Prepared according to the method described by Bougault (25).

TABLE VI.—BACTERIOSTATIC ACTION OF β-(*o*-HYDROXYBENZOYL)-ACRYLIC ACID IN THE PRESENCE OF CYSTEINE HYDROCHLORIDE OR P.A.B.A.

Antibacterial Agent (1:5000 in 10% EtOH)	Molar Equivalents of Cysteine HCl or P.A.B.A. to Antibacterial Agent	Highest Dilution Causing Inhibition of Growth	
		S. aureus	*E. typhosa*
O.H.B.A.[a]	...	1:40,000	1:40,000
O.H.B.A. + Cysteine HCl	0.5	1:40,000	1:40,000
O.H.B.A. + Cysteine HCl	1.0	1:40,000	1:40,000
O.H.B.A. + Cysteine HCl	2.0	1:40,000	1:40,000
O.H.B.A. + Cysteine HCl	3.0	1:40,000	1:40,000
O.H.B.A. + P.A.B.A.	0.5	1:40,000	1:40,000
O.H.B.A. + P.A.B.A.	1.0	1:40,000	1:40,000
O.H.B.A. + P.A.B.A.	2.0	1:40,000	1:40,000

[a] β-(*o*-Hydroxybenzoyl)-acrylic acid.

mined in mice. Upon oral administration there was no death up to 1600 mg./Kg. Intraperitoneally, the LD_{50} was 75 mg./Kg.

FOOTNOTES TO TABLE IV

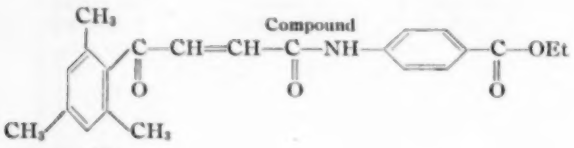
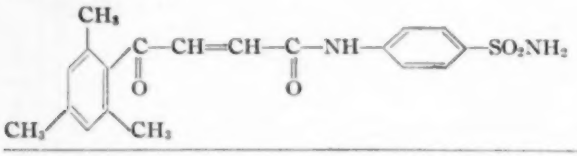
* No activity in the lowest dilution tested (1:10,000).
[a] M. p. reported, 116° (21).
[b] B. p. reported, 128° (2 mm.) (22).
[c] M. p. reported, 148° (23).
[d] Calcd. for $C_{17}H_{13}O_4N$: N. E., 157. Found: N. E., 159.
[e] Calcd. for $C_{16}H_{14}O_7Hg$: Hg, 40.5. Found: Hg, 40.61.
[f] Calcd. for $C_{19}H_{20}O_6S$: S, 8.9. Found: S, 8.8.
[g] Calcd. for $C_{15}H_{15}O_6NS$: S, 10.22. Found: S, 10.22, 10.35.
[h] Calcd. for $C_{18}H_{17}O_6N$: C, 62.97; H, 4.99; N. E., 171. Found: C, 62.65; H, 5.01; N. E., 167.
[i] Calcd. for $C_{14}H_{17}O_6NS$: S, 9.78. Found: S, 9.29, 9.35.

DISCUSSION

The acids listed in Table II afford an opportunity to make some generalizations about the relationship of chemical structure to antibacterial activity for this series of compounds. However, before attempting this it would be expedient to discuss the chemical properties of the parent compound, β-benzoyl acrylic acid, in order to gain a better insight into the possible mode of action of this series of antibacterial agents.

The β-aroyl acrylic acids contain an ethylenic linkage which shows remarkable ease of addition. Addition compounds of diazomethane, methanol,

TABLE VII.—EFFECT OF SERUM ON BACTERIOSTATIC ACTIVITY

Compound	Highest Dilution Causing Inhibition of Growth of *S. aureus*	
	No Serum	10% serum
	1:1,600,000	Inactive at 1:400,000
	1:800,000	Inactive at 1:400,000

and hydrogen chloride to β-(*p*-brombenzoyl)-acrylic acid have been prepared (26). In this laboratory the easy addition of cysteine and *p*-aminobenzoic acid to the β-benzoyl acrylic acid compounds has been observed. The resulting saturated ketones are no longer effective antibacterial agents. Thus it would appear that in conformance with the postulate of Geiger and Conn (1) these compounds owe their activity against bacteria to their ability to add some essential metabolite or enzyme system across the highly active α,β-unsaturated ketonic structure.

According to the work of Kohler (27), α,β-unsaturated ketones are converted into saturated ketones by 1,4-addition. In the case of β-benzoyl acrylic acid it was shown that HA reagents add to the conjugated system containing the keto group (28).

Although α,β-unsaturated carbonyl systems show a marked tendency to undergo conjugate addition there are many instances in which one of the multiple bonds functions independently. Kohler (29) has pointed out that nearly all reactions involving 1,2-addition to carbonyl are reversible, whereas the products formed by 1,4-addition (except with organometallic compounds) undergo rearrangement into saturated carbonyl compounds that are still capable of undergoing 1,2-addition. Under these conditions the products ultimately isolated do not represent the relative rates of 1,2- and 1,4-addition but merely the relative stability of the substances or the position of the equilibrium in the particular environment.

The following reactions could conceivably occur between an HA compound (in this case HA = some bacterial enzyme system or some essential metabolite) and the β-aroyl acrylic acids.

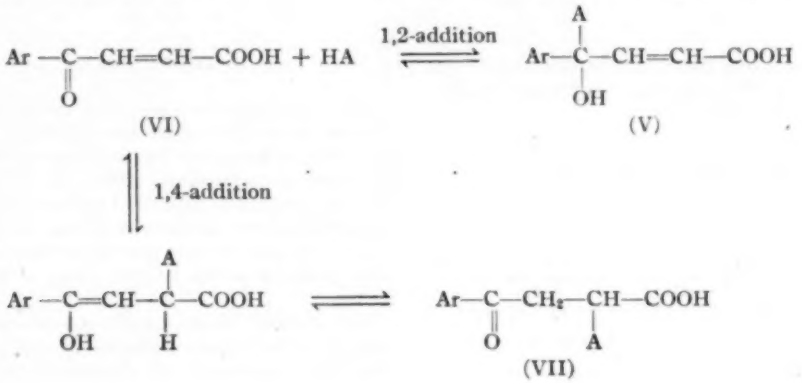
TABLE VIII.—THE BACTERIOSTATIC ACTION OF THE *p*-CARBETHOXY ANILIDE OF β-MESITOYL ACRYLIC ACID

Organism	Highest Dilution Causing Inhibition of Growth
Staphylococcus aureus No. 2092	1:1,000,000[a]
Staphylococcus albus No. 2	1:1,000,000[a]
Streptococcus hemolyticus No. 2	1:2,000,000[b]
Streptococcus viridans No. 4	1:5,000,000[b]
Diplococcus pneumoniae Type II	1:5,000,000[b]
Hemophilus influenzae No. 2	1:2,000,000[b]

[a] *Medium.*—F. D. A. broth.
[b] *Medium.*—Beef infusion broth.

Of the acids listed in Table II only five have an activity over 1:100,000 against *S. aureus* and of these only the triethyl benzoyl acrylic acid (No. 4) shows a similar efficacy against *E. typhosa*. It is difficult to pick out any characteristic structural feature in the substituted benzoyl acrylic acids, which accounts for the enhancement in antibacterial activity. However, in the case of β-mesitoyl acrylic acid (No. 3) and β-(2,4,6-triethyl benzoyl)-acrylic acid (No. 4) it would appear that steric hindrance might play a role. Furthermore, one is tempted to attribute the enhancement to a purely geometric effect because the larger alkyl group is much more effective.

The presence of the alkyl groups in both ortho positions to the unsaturated side chain would inactivate the carbonyl group. Thus, for example, the keto group of acetomesitylene is inactive toward the usual carbonyl reagents (30). As the activity of the carbonyl group decreases the amount of 1,4-



addition increases (31), consequently in the case of the sterically hindered β-aroyl acrylic acids the amount of the unstable adduct (V) formed by 1,2-addition would be negligible. This would result in a rapid formation of the more stable adduct (VII) and thus account for the greater antibacterial activity.

With the other three active acids (Nos. 24, 26, and 27) it is difficult to arrive at any definite relationship between structure and activity. However, it may be that greater solubility in the bacterial cell accounts for their inhibitory power in higher dilution.

The esters and amides are much more effective than the corresponding acids. Here again it could be a matter of better solubility in the bacterial cell.

From the results in Table III it would appear that there is no advantage in replacing the benzene ring by a thiophene, carbazole, or dibenzofuran ring.

All the compounds listed in Table IV, with the exception of two, show no inhibitory properties in the lowest dilution tested. The activity of the piperidine adduct (No. 66) is difficult to explain. However, the mercuric acetate derivative (No. 69) most likely owes its antibacterial action to the mercuric acetate group. This is in conformance with the well-known proficiency of the organomercurials as bacteriostatic agents.

The lack of activity in the other β-benzoyl propionic acid derivatives is evidence for the essentiality of the α,β-unsaturated system.

The first three compounds in Table V (Nos. 78, 79, and 80) show some promise. We are unable to explain the lack of activity against *S. aureus* by the latter two.

SUMMARY

The bacteriostatic properties of a series of ring-substituted β-aroyl acrylic acids, esters, and amides are described. The most effective compounds contain alkyl groups in the ortho positions with respect to the unsaturated side chain.

The esters and amides are more effective than the corresponding acids.

The antibacterial potency of β-(*o*-hydroxybenzoyl)-acrylic acid is not nullified by the presence of cysteine hydrochloride or *p*-aminobenzoic acid.

The bacteriostatic potency of two of the most effective compounds was greatly reduced in the presence of serum.

Saturation of the ethylenic linkage in the side chain brings about a loss in antibacterial activity.

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Pharmacognostical and Chemical Studies of Indian Belladonna, *atropa acuminata* Royle ex Lindley*

By HEBER W. YOUNGKEN† and WILLIAM E. HASSAN, JR.‡

Comparative gross anatomical and histological studies are reported on the roots, stems, leaves, flowers, and fruits of *Atropa acuminata* and of *Atropa Belladonna*. A chemical study of the alkaloidal content of both species is reported in which it is shown that Indian Belladonna possesses a higher alkaloidal content than the European species.

IN THE British Pharmacopœia of 1932, add. 7 (1), along with *Atropa Belladonna*, we find *Atropa acuminata* mentioned as an additional source of Belladonna. The latter is an Indian species and has been gathered in large amounts for use in the British Commonwealth of Nations. A number of pharmacognosists and chemists have reported on various phases of Indian Belladonna but the reports have been scattered and some of the literature pertaining to the taxonomy and alkaloidal constituents has been chaotic. The purpose of this paper is to straighten out this confusion by attempting to confirm some of the results hitherto reported and adding new facts concerning the structure, constituents, and taxonomy of Indian Belladonna. Representative specimens consisting of herbarium sheets and preserved materials have been permanently deposited in the herbarium of the Massachusetts College of Pharmacy.

TAXONOMY

The taxonomic history of this plant may be traced back to the middle of the eighteenth century. Two names have been applied to it, *Atropa acuminata* Royle and *Atropa lutescens* Jacquemont but both of these are equated to *Atropa Belladonna* L. in the Index Kewensis. The name *Atropa acuminata* was used by Royle in his *Illustrations of the Botany of the Himalaya* without a description and is a *nomen nudum*.

Atropa lutescens Jacquemont has been used by Aitchinson (2) in his account of the flora of the Kurrum Valley, by Watt (3) in his *Dictionary of the Economic Products of India*, and by several other pharmaceutical writers. There is no mention of an *Atropa* in Jacquemont's *Voyage Dans L'Inde Botanique*, 1884, and he does not seem to have validated his manuscript name elsewhere by a description.

Clark (4) cites *Atropa lutescens* Jacquemont together with *Atropa acuminata* Royle as synonymous with *Atropa Belladonna* L. in Hook. f., *Flora of British India*. (4), though *Atropa acuminata* is validated much earlier by Lindley in the *Journal of the Horticultural Society of London* (5) where a brief description is given of plants raised in the Society's garden from seeds sent by Capt. William Munroe in 1845.

Tschirch, in his *Handbuch der Pharmakognosie*, 1923 (6), uses the combination *Atropa acuminata* Meirs. In this he is followed by Hegi in *Flora von Mittel-Europa*, 1927, but Meirs himself attributes the name to Royle. The synonymy and the relevant literature pertaining thereto are as follows:

Atropa acuminata Royle
"Bot. Himal.," 279, (1839), *nomen nudum*.
Atropa lutescens Jacquemont, *nomen nudum*.
Atropa acuminata Royle ex Lindley
J. Hort. Soc. (*London*), I, 306(1846).
Atropa acuminata Royle ex Meirs
J. Bot., I, 138(1849) and "Illust. S. Amer. Plants," ii, app. 5, 5, T76, 1857.
Atropa acuminata, Meirs
Tschirch, A., "Handbuch der Pharmakognosie," III (i), 268, 1923.
Atropa lutescens, Jacquemont ex Clarke
Hooker fil. "Flora of British India, Vol. IV, 241(1885).
Atropa acuminata Meirs ex Hegi
"Flora von Mittel-Europa," Vol. V (4), 2565, (1927).

It is quite evident that the nomenclature for Indian Belladonna has been chaotic.

MATERIALS

The materials for this investigation consisted of the entire plants of *Atropa Belladonna* L. and *Atropa acuminata* Royle ex Lindley. The seeds of the European variety were gathered from the fruits of authentic plants of *Atropa Belladonna* grown in the College Medicinal Plant Garden. Those of the Indian variety were obtained from Dr. A. F. Sievers of the United States Department of Agriculture Station, Beltsville, Maryland.

The seeds were first planted in flats and the seedlings kept in a local greenhouse until they were transplanted into individual flower pots. When they were large enough, some of the plants were again transplanted into the College Medicinal Plant Garden in Jamaica Plain, Boston, Massachusetts, and others were planted at Brockton, Massachusetts.

Specimens of the roots, stems, leaves, and flowers were gathered at various times during their growth, examined macroscopically and microscopically, and the surplus preserved materials have been deposited in the Herbarium of the Massachusetts College of Pharmacy.

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DESCRIPTION OF THE PLANT

Indian Belladonna (Fig. 1) as grown in Boston, Massachusetts, is a bushy perennial herb with dichotomously branched stems. The plant attains an average height of 3 to 4 ft. The leaves (Figs. 1 and 2) vary in color from green to olive-green and measure up to 30 cm. in length and up to 12 cm. in width. They are oblong-elliptical, tapering gradually at both the apex and the base, whereas the leaves which are located close to the flowering tops are more pointed at the apex and less tapering at the base. On the flowering branches, the leaves occur in pairs, nearly opposite, the lowest one having a single flower in its axil. The slightly higher one exhibits a secondary branching system with 2 to 4 flowers together in a bracteate cyme. The angle which the second and third lateral veins of the leaves make with the midrib is usually from 30 to 40 degrees.

Fig. 1.—*Atropa acuminata* Royle ex Lindley. Portion of plant grown in medicinal plant garden of the Massachusetts College of Pharmacy showing leaf and flowering branches and a larger leaf from another branch of the same plant. × 1/2.

The flowers occur either solitary, or in groups of 2 to 4, in bracteate cymes. They are axillary and drooping and possess a calyx which is deeply 5-cleft and a yellow to greenish yellow, campanulate to tubular-campanulate corolla with 5 broad, spreading, recurved lobes. There are 5 epipetalous stamens, an exserted style, and a knob-like stigma. The ripe fruit is a sub-globular black berry with a persistent calyx.

The rootstock is hard and woody, from 3 to 5 cm. in width, and the first year root, which is comparatively small in bulk, does not exceed 2 cm. in width. Upon drying, the root becomes wrinkled longitudinally. The fracture is tough and does not always break with a short mealy fracture as is characteristic of the root of the European Belladonna.

PALISADE RATIO OF INDIAN AND EUROPEAN BELLADONNA

Zörnig and Weiss (7) in their paper on certain compositaceous leaves stated that the average number of palisade cells beneath an upper epidermal cell was diagnostically valuable. They also stated that the number of palisade cells per unit area increased successively from the base of the leaf to the upper portion of the apex but since there was now a corresponding diminution in the area of the epidermal cells, the ratio remained almost constant.

Wallis and Dewar (8), later, in their study of the comparative anatomy of the Barosmas, concluded that variation in the palisade ratio is independent of the position on the leaf. Furthermore, they were the first to use the term "palisade ratio" stating in their publication that the total number of palisade cells beneath four upper epidermal cells, when divided by four, gave the recorded figure for the palisade ratio, this being the term and method employed in this paper to express the average number of palisade cells beneath an upper epidermal cell.

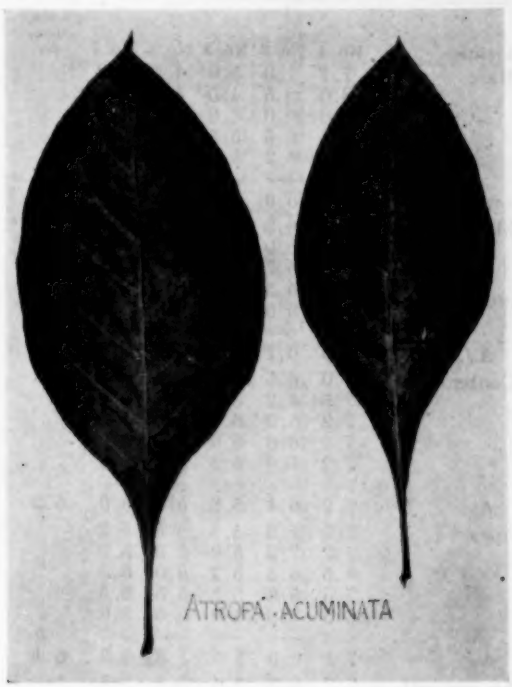

Fig. 2.—Leaves of *Atropa acuminata* Royle ex Lindley. × 1/2.

Wallis and Forsdike (9), in their investigation of the palisade ratio of *Atropa Belladonna Scopolia*

carniolica, and *Solanum nigrum*, concluded that the palisade ratio does not change with the age of the leaf, the habitat or from year to year within any definite species.

Dewar (10–12) has reported on the palisade ratios of *Digitalis Thapsi*, *D. lutea* , and *D. lanata*.

Feinstein and Slama (13) found that the palisade ratio could be used in differentiating the leaves of *Atropa Belladonna* from those of official Stramonium, Hyoscyamus, and Digitalis.

George (14), in his study of the ratio of the Indian and the European varieties of Belladonna, concluded that *Atropa acuminata* had a palisade ratio which falls within the range of 6.9 to 9.2, whereas *Atropa Belladonna* falls within the range of 5.0 to 7.0.

From the foregoing discussion, it seemed possible that the palisade ratio might prove to be of pharmacognostic value in aiding in the differentiation of these two species of Belladonna.

TABLE I.—PALISADE RATIOS OF ATROPA BELLADONNA LEAVES

Location	No. 1	No. 2	No. 3	No. 4	No. 5	Location, Av.
Base	7.7	7.0	5.0	4.5	5.2	
	8.0	5.5	4.5	5.2	4.5	
	7.0	6.0	6.0	5.0	4.8	
	7.5	6.5	5.5	4.5	5.2	
	8.0	5.2	5.2	6.0	5.0	
Av.	7.6	6.0	5.2	5.0	4.9	5.7
Margin	8.5	6.5	5.0	6.5	5.8	
	9.0	6.0	5.2	5.5	6.7	
	8.8	6.7	5.5	5.7	5.5	
	9.2	5.5	5.7	5.0	5.5	
	9.0	6.0	4.5	5.4	5.0	
Av.	8.9	6.1	5.2	5.6	5.7	6.3
Center	7.0	6.5	5.5	6.2	6.7	
	7.8	6.2	5.5	6.0	6.2	
	7.2	6.5	6.5	5.5	5.5	
	7.0	6.0	6.0	5.5	5.2	
	7.2	7.0	5.8	6.0	6.0	
Av.	7.2	6.4	5.8	5.8	6.0	6.2
Apex	7.0	7.5	5.5	6.5	5.2	
	7.7	7.2	5.0	5.5	5.0	
	6.5	6.5	5.2	6.0	6.0	
	7.5	6.6	5.5	5.5	5.5	
	6.5	7.0	6.0	5.5	5.0	
Av.	7.1	6.9	5.4	5.8	5.3	6.1
Av. per leaf	7.7	6.3	5.4	5.5	5.5	

Notes: 1. Average palisade ratio for the species 6.1.
2. Lowest recorded palisade ratio 4.5.
3. Highest recorded palisade ratio 9.2.
4. Mean average 6.9.

The method followed was to cut out pieces 2–3 mm. square, with the aid of a sharp blade, from the apex, margin, center, and base. These segments were then placed in a clearing solution which consisted of 50 Gm. of chloral hydrate dissolved in 20 cc. of water and gently heated upon a water bath for approximately fifteen minutes. The material was then removed and mounted in Berlese's Modified Mountant. In each determination, the number of palisade cells under any four adjacent epidermal cells, when divided by four, represented the palisade ratio and in no case was any palisade cell included where more than one-half of its area lay outside of the epidermal cell.

The results of this study are recorded in Tables I and II which are constructed to show the palisade ratio for each leaf, for the specific location, and for the species.

VEIN ISLET NUMBER DETERMINATION

Eames and MacDaniels (15) define a vein islet as "the ultimate divisions of the conducting strands completely or partially encircling minute areas of the photosynthetic tissues with which they are in close contact."

The term "Vein Islet Number" was coined by Levin who defined it as "the number of vein islets in a square millimeter of leaf tissue."

TABLE II.—PALISADE RATIOS OF ATROPA ACUMINATA LEAVES

Location	No. 1	No. 2	No. 3	No. 4	No. 5	Location, Av.
Base	8.5	8.0	5.0	7.5	8.2	
	8.0	7.5	5.8	7.0	7.5	
	7.7	7.0	6.5	7.6	7.8	
	7.5	7.5	6.5	8.0	8.0	
	8.0	7.7	5.5	8.1	8.0	
Av.	7.7	7.5	5.8	7.6	7.9	7.3
Margin	11.5	8.5	6.0	8.2	8.2	
	10.2	8.5	6.8	8.0	8.9	
	10.0	9.0	7.5	8.6	9.8	
	9.8	8.6	8.0	9.0	8.5	
	9.5	9.0	8.2	9.5	8.8	
Av.	10.2	8.7	7.3	8.4	8.8	8.7
Center	9.0	8.5	8.5	8.2	8.7	
	9.8	8.2	7.5	8.0	8.2	
	8.8	8.5	9.5	9.5	7.5	
	8.8	7.0	7.0	8.5	6.6	
	8.4	8.0	7.8	9.0	7.0	
Av.	8.9	8.0	8.0	8.6	7.6	8.2
Apex	12.4	9.0	8.8	9.2	8.8	
	11.6	8.5	7.8	8.6	8.5	
	11.1	8.7	9.2	9.1	7.8	
	10.8	8.3	8.0	8.7	7.9	
	10.7	8.2	8.8	8.5	8.2	
Av.	11.6	8.5	8.3	8.5	8.2	9.0
Av. per Leaf	9.6	8.2	7.3	8.3	8.1	

Notes: 1. Average palisade ratio 8.3.
2. Lowest palisade ratio 5.0.
3. Highest palisade ratio 12.4.

In our examination, a method proposed by Zufall and Burlage (16) was employed, using a slight modification. Pieces of the leaf material approximately 4 mm. square were cut from the apex, center, margin, and base. After clearing with chloral hydrate solution, the sections were mounted on glass slides in the clearing medium and examined microscopically.

The area of the vein islet may be determined by counting the number of vein islets within the field of the 16 mm. objective. The number of vein islets

within the field divided by the area of the field, in mm., gives the average area of the vein islet.

The 16-mm. objective gave a field whose area was 1.98 sq. mm. This is close enough to the whole number 2, so that the "Vein Islet Number" can be easily calculated. The vein islets in a field are counted and the "Vein Islet Number" is obtained by dividing this figure by two. The data obtained are recorded in Tables III–VII.

TABLE III.—VEIN ISLET NUMBER DETERMINATIONS OF THE LEAF APEX OF ATROPA ACUMINATA*

Leaf No.	Field No.	Av. V. I. N.	Leaf Position, Av.
1	1	14	
1	2	11	
1	3	12	
1	4	12	14.7
2	1	15	
2	2	11	
2	3	13	
2	4	10	12.2
3	1	12	
3	2	10	
3	3	12	
3	4	6	10.0
4	1	10	
4	2	9	
4	3	9	
4	4	7	8.7
5	1	10	
5	2	9	
5	3	9	
5	4	8	9.0

* See Fig. 2.

TABLE IV.—VEIN ISLET NUMBER DETERMINATIONS OF THE LEAF CENTER OF ATROPA ACUMINATA

Leaf No.	Field No.	Av. V. I. N.	Leaf Position, Av.
1	1	11	
1	2	10	
1	3	10	
1	4	8	9.7
2	1	11	
2	2	10	
2	3	12	
2	4	10	10.7
3	1	10	
3	2	10	
3	3	10	
3	4	9	9.7
4	1	10	
4	2	10	
4	3	9	
4	4	9	9.5
5	1	10	
5	2	9	
5	3	8	
5	4	9	9.0

STOMATAL INDICES

We have investigated the stomatal indices of the Indian and the European varieties of Belladonna using the formula advocated by Salisbury (17) and the procedure used by George (19) for Senna.

The results of our investigation are in accord with those obtained by Rowson (18) in his investigation on the English-grown plant. The results of our investigation are as follows:

Upper surface of Indian Belladonna....	3.4
Upper surface of European Belladonna..	2.9
Lower surface of Indian Belladonna....	17.6
Lower surface of European Belladonna..	17.6

TABLE V.—VEIN ISLET NUMBER DETERMINATIONS OF THE LEAF MARGIN OF ATROPA ACUMINATA

Leaf No.	Field No.	Av. V. I. N.	Leaf Position, Av.
1	1	13	
1	2	14	
1	3	13	
1	4	14	13.5
2	1	13	
2	2	12	
2	3	11	
2	4	10	11.5
3	1	10	
3	2	13	
3	3	12	
3	4	10	11.2
4	1	12	
4	2	10	
4	3	12	
4	4	11	11.2
5	1	12	
5	2	11	
5	3	12	
5	4	10	11.2

TABLE VI.—VEIN ISLET NUMBER DETERMINATIONS OF THE LEAF BASE OF ATROPA ACUMINATA

Leaf No.	Field No.	Av. V. I. N.	Leaf Position, Av.
1	1	9	
1	2	7	
1	3	7	
1	4	7	7.5
2	1	8	
2	2	12	
2	3	9	
2	4	10	9.7
3	1	10	
3	2	7	
3	3	8	
3	4	7	8.0
4	1	10	
4	2	7	
4	3	8	
4	4	7	8.0
5	1	10	
5	2	10	
5	3	11	
5	4	10	10.0

TABLE VII.—SUMMARY OF V. I. N. DETERMINATIONS

Leaf av. no. 1.....	11.3
2.....	13.5
3.....	10.2
4.....	9.3
5.....	9.8
Species av.....	10.0

A COMPARATIVE STUDY OF THE FLORAL PARTS OF INDIAN AND EUROPEAN BELLADONNA

The flowers for this study came from the first year plant soon after the opening of the calyx and corolla. The measurements were made upon 25 freshly gathered flowers with a metric rule.

The calyx of the Indian variety is green to yellowish green and glandular-pubescent. The sepals are joined to form a cup from 0.4 to 0.7 cm. long. The lobes of the calyx measure from 1.0 to 2.1 cm. in length and are triangular to ovate-triangular, the apices are acuminate, and the entire margin recurved. Estivation is valvate to imbricate.

The calyx of the European variety compares favorably to the above description with the exception that the calyx here is a true green at all times. The sepal cup measures from 0.3 to 0.6 cm. and the lobes of the calyx are from 1.0 to 1.5 cm. in length.

The corolla of *Atropa acuminata* is campanulate to tubular campanulate and from 1.5 to 2.8 cm. in length and from 0.5 to 1.3 cm. in width at the mouth. The lobes are from 0.3 to 0.7 cm. in length and are semielliptical with entire rolled margins. The corolla is bright yellow to greenish yellow in color with purplish veins in the lower portion and a dull brownish margin around the mouth.

The European variety exhibits a campanulate corolla measuring from 2.4 to 3.3 cm. in length and from 1.1 to 1.4 cm. in diameter at the mouth. The lobes vary slightly in that they measure from 0.4 to 0.7 cm. in length. The corolla is a vivid purple merging into a pale green where it enters the calyx. The under surface is velvety in the upper part and glossy where green. Pigmentation of the veins is a dark purple.

The style of the Indian variety is filiform and solid, and ranges in length from 1.6 to 2.0 cm. It rises from the apex of the ovary and varies in color from yellowish green to yellow-white. It is slightly arched and is terminated by a capitate, obscurely bilobed stigma which is yellow-green in color and quite fleshy.

The style of European Belladonna is longer than that of the Indian variety, the length varying from 2.3 to 2.5 cm.

The stamens of the Indian variety are epipetalous and measure from 1.0 to 1.5 cm. in length. Greenish yellow filaments are adherent to the base of the corolla for a very short distance over the point of adhesion. Numerous long trichomes grow from the corolla and the filaments. The filaments are hooked beneath the anthers which are about 0.2 cm. long, dorsifixed, and dehiscing introrsely by two longitudinal splits; the walls contract to expose a mass of pale yellow pollen.

The stamens of the European Belladonna are epipetalous and measure 1.5 to 2.0 cm. in length, and the anthers are approximately 0.3 cm. in length.

The ovary of the Indian variety is sphero-conical and about 0.3 cm. in length and from 0.3 to 0.4 cm. in diameter at the widest part. It is superior, bicarpellary, and bilocular with two longitudinal depressions externally, corresponding in position to the edges of the septum. The nectary is in the form of an annular swelling at the base. Placentations seem to be axile. The placentae and the small ovules completely filled the loculi. No significant variation was noted between the ovaries of the two plants.

STEM HISTOLOGY OF ATROPA ACUMINATA

Sections of the stem 2.5 to 3.0 mm. in diameter (Fig. 3) presented the following microscopical characteristics.

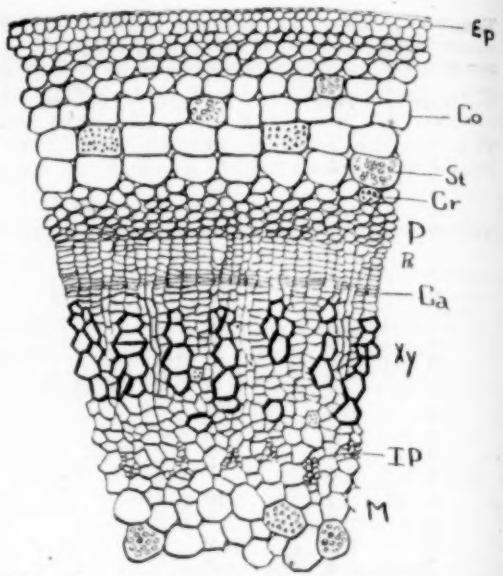

Fig. 3.—*Atropa acuminata*. Transverse section of stem. *Ep*, epidermis; *Co*, cortex; *St*, starch; *Cr*, microcrystals; *P*, pericycle; *Ph*, phloem; *Ca*, cambium; *Xy*, xylem; *IP*, internal phloem; *M*, pith.

The epidermis consisted of small square to slightly tangentially elongated epidermal cells, as viewed in cross section, the cells polygonal to irregular-shaped in surface section, with a striated cuticle and beaded vertical walls, and showing numerous uniseriate, nonglandular hairs, many up to 5-celled, a few up to 6-celled, also numerous glandular hairs with up to 5-celled, uniseriate stalks and small subspherical to spherical heads. Numerous stomata of the solanaceous type were present, the stomates being predominantly oval in shape.

The cortex consisted of approximately 6 layers of chlorophylloid collenchyma.

The pericycle was narrow and consisted of 2 or 3 layers of starch and microcrystal-bearing parenchyma in which were scattered pericyclic fibers with thin, nonlignified walls and broad lumina.

The sections showed many bicollateral fibrovascular bundles with narrow external and internal phloem and broader xylem zones, the xylem exhibiting narrow wood rays and wood wedges, the latter consisting chiefly of tracheary tissue and a limited number of wood fibers.

The pith is composed of richly protoplasmic, pitted parenchyma, many of the cells containing masses of microcrystals.

Sections of the stem 10 mm. or more in diameter exhibited an epidermis which differed from the

younger stems in showing relatively few hairs, larger epidermal cells, the latter with nonbeaded or indistinctly beaded vertical walls, many containing pink to purple anthocyanin. The cortex is broader, mostly parenchymatous with larger cells. The pericyclic fibers are lignified and thicker-walled. The xylem wedges are deeper than in younger portions of the stem and show a considerable increase of wood fibers. The internal phloem exhibits larger islets of sieve. The tracheae and the trachieds are of the simple pitted, bordered pitted, reticulate, and spiral types occurring in the primary xylem. The pith is broad consisting of pitted parenchyma, the cells of which are mostly larger than in young stems and, in sections 10 mm. in diameter, show beginning disintegration in the central area. A number of scattered pith cells contained microcrystals. Sections of the stem cut in midsummer showed a green pith, numerous chloroplastids, and small starch grains. The medullary rays possess lignified walls in older stem parts.

COMPARATIVE HISTOLOGY OF THE ROOTS OF THE INDIAN AND EUROPEAN VARIETIES OF BELLADONNA

During the course of this comparative histology, the work of Melville (20) was consulted frequently and the figures stated here are within or relatively close to the range of his findings.

The cork of *Atropa acuminata* is similar to that of *Atropa Belladonna* except that the cells in surface view are larger. The dimensions from the measurements of approximately 200 cells are: length minimum 30 μ, maximum 200 μ, with the majority lying between 50 and 130 μ. Melville states that the average length per cell is 95 μ. The width showed a minimum of 20 μ and a maximum of 86 μ with the majority lying between 32 and 56 microns. The stated general width average per cell was 44 μ.

For *Atropa Belladonna* L., the dimensions for a similar number of measurements were such that the average for the length was 72.5 μ and for the width 39.5 μ.

Melville (20) in his investigation stated that the cork cells per sq. mm. calculated from counts of 25 fields of about 0.1 sq. mm. were for the Indian root a minimum of 167 to a maximum of 400, the majority numbering between 225 and 345. The mean is 285. A similar count on *Atropa Belladonna* yielded a mean of 470. A statistical analysis of the figures shows that the mean of 10 such counts will fall within 347 to 593 cells per sq. mm. for *Atropa Belladonna* and within the range of 227 and 344 cells per sq. mm. for *Atropa acuminata*. One may then consider that if the mean of 10 such counts is taken, a value above 347 indicates *Atropa Belladonna* and below, *Atropa acuminata*.

The phelloderm consists of about 10 layers of cells differing from those of *Atropa Belladonna* in that some contain a reddish brown resinous substance in addition to the starch and crystals.

The secondary phloem consists of up to 50 layers of cells, mainly starch-and microcrystal-bearing parenchyma and radial groups of sieve tissue similar to that of *Atropa Belladonna*. On the outer border are a few isolated fibers with associated groups of collapsed sieve tissue; scattered throughout the parenchyma are numerous lignified fibers and fibrous cells. The fibers are tapering and pointed, their walls are striated, thick, and show simple pitting. The fibrous cells appear as axially elongated irregular prisms, with the walls moderately thick and with a few relatively small simple pits. They average approximately 300 μ in length and 28 μ in diameter.

The cambium consists of up to 3 layers of collapsed cells, and is similar to that of *Atropa Belladonna*.

The secondary xylem in young roots is composed of starch- and crystal-bearing parenchyma with strands of up to 10 vessels and associated tracheids, fibers, and lignified parenchyma. The strands located close to the cambium are radially arranged forming a hollow cylinder, but are more scattered as they approach the center, an arrangement similar to that of *Atropa Belladonna*.

The primary xylem is a central, solid, di-arch strand similar to that of *Atropa Belladonna*.

The medullary rays at the cambium average up to 7 per mm.; in tangential view they are elongate, spindle-shaped and up to 20 cells high and 1 to 5 cells wide. The cells are similar to those of *Atropa Belladonna* or are often more axially elongated. The average length ranges between 80 and 180 μ.

Starch occurs abundantly in all of the parenchymatous tissues, the individual grains being similar to those of *Atropa Belladonna*. The diameter of 100 measured grains did not exceed 15.0 μ. More measurements are contemplated.

Sphenoidal microcrystals occur in all of the parenchymatous tissues of both species.

A COMPARATIVE STUDY OF THE BIOSYNTHESIS OF HYOSCYAMINE

Cromwell (21), during his studies on the biosynthesis of various Solanaceous alkaloids, discovered that alkaloids in general are synthesized from prod-

TABLE VIII.—ATROPA ACUMINATA

Solution Injected	Leaf		Root	
	Control	Experimental	Control	Experimental
Potassium Nitrate	0.342	0.335	0.525	0.530
Ammonium Sulfate	0.340	0.366	0.528	0.538
Glycine	0.338	0.357	0.524	0.548
Arginine	0.345	0.379	0.525	0.544
Putrescine[a]	0.346	0.483	0.528	0.586
Methylamine[a]	0.341	0.349	0.526	0.548
Dimethylamine[a]	0.342	0.350	0.524	0.545
Trimethylamine[a]	0.345	0.356	0.525	0.539

TABLE IX.—ATROPA BELLADONNA

Solution Injected	Leaf		Root	
	Control	Experimental	Control	Experimental
Potassium Nitrate	0.320	0.324	0.515	0.520
Ammonium Sulfate	0.322	0.348	0.518	0.527
Glycine	0.328	0.342	0.514	0.538
Arginine	0.325	0.358	0.515	0.534
Putrescine[a]	0.325	0.452	0.518	0.563
Methylamine[a]	0.320	0.331	0.516	0.538
Dimethylamine[a]	0.324	0.326	0.514	0.535
Trimethylamine[a]	0.325	0.342	0.515	0.529

[a] Dihydrochloride.

The values given represent the average of 3 injections on separate plants in the seventh month of their first year's growth.

Figures represent Gm. of alkaloid per 100 Gm. of dry weight.

ucts of carbohydrate and protein breakdown. In a report published in 1943, Cromwell (21) showed that the alkaloid hyoscyamine could be biosynthesized in *Atropa Belladonna* by use of the various amino acids and related amines.

Using Cromwell's method, the European and the Indian varieties of Belladonna were subjected to the various amino salts and other chemicals for the purpose of comparing the biosynthesis of the alkaloid hyoscyamine in the two species.

A solution containing 2% glucose and 0.25% of the active substance was placed in a one-liter aspirator flask approximately 2.5 to 3.0 ft. above the plant and the solution conveyed to the cut end of the stem through a rubber tubing provided with a screw clip to serve as a shut-off. With good transpiration, as much as 100 cc. of the solution passed through the plants in a single day. The injection was allowed to carry on for a week. The plant was then cut, dried at room temperature, and chemically assayed.

The comparative results of this experiment are given in Tables VIII and IX.

CONSTITUENTS

When the British Pharmacopœial Commission admitted Indian Belladonna to the British Pharmacopœia to serve as an alternative to the European variety in galenical preparations, a controversy arose as to the reliability of the official method of assay. This method did not insure more than the partial removal of the known volatile bases, therefore causing a variation in the final alkaloidal determination.

Evers and Caines (22) and Markwell and Walker (23), through numerous chemical assays, have concluded that *Atropa acuminata* contained a higher per cent of alkaloid and a far greater per cent of volatile bases.

The volatile bases have therefore been subjected to chemical analysis by various authors. It has been stated that the base contains a variable amount of tropine, pyridine, N-methylpyrroline, N-methylpyrrolidine, and tetra-methyldiaminobutane.

The following method of assay was employed in the chemical investigation of the Belladonnas:

TABLE X.—ATROPA ACUMINATA BERRIES

Sample No.	Drying Method	Temp., ° C.	Wt., Gm.	Alkaloid, %
1	Infrared	145	25	0.060
2	Infrared	145	25	0.055
3	Infrared	145	25	0.058
4	Infrared	55	25	0.296
5	Infrared	55	25	0.308
6	Infrared	55	25	0.305
7	Infrared	55	25	0.305
8	Infrared	55	25	0.306
9	Natural	22	25	0.309
10	Natural	22	25	0.306
11	Natural	22	25	0.303
12	Natural	22	25	0.306

Samples 1, 2, and 3 were dried under two infrared lamps, one set 10 inches above the sample and the other 10 inches below. Drying was allowed to go on for one hour.

Samples 4, 5, 6, 7, and 8 were dried under one infrared lamp placed at a distance of 24 inches above the fruits. Drying was allowed to continue for a period of two hours.

Samples 9, 10, 11, and 12 were spread out and allowed to dry at room temperature. The length of time for drying was approximately six to eight weeks.

TABLE XI.—ATROPA BELLADONNA BERRIES

Sample No.	Drying Method	Temp., ° C.	Wt. Gm.	Alkaloid, %
1	Infrared	145	25	0.051
2	Infrared	145	25	0.050
3	Infrared	145	25	0.051
4	Infrared	55	25	0.286
5	Infrared	55	25	0.295
6	Infrared	55	25	0.296
7	Infrared	55	25	0.296
8	Infrared	55	25	0.296
9	Natural	22	25	0.300
10	Natural	22	25	0.298
11	Natural	22	25	0.298
12	Natural	22	25	0.298

Samples 1, 2, and 3 were dried under two infrared lamps, one set 10 inches above the sample and the other 10 inches below. Drying was allowed to go on for one hour.

Samples 4, 5, 6, 7, and 8 were dried under one infrared lamp placed at a distance of 24 inches above the fruits. Drying was allowed to continue for a period of two hours.

Samples 9, 10, 11, and 12 were spread out and allowed to dry at room temperature. The length of time for drying was approximately six to eight weeks.

TABLE XII.—BERRY AND CALYX OF ATROPA ACUMINATA[a]

Sample No.	Temp., ° C.	Wt. Gm.	Alkaloid, %
1	22	25	0.55
2	22	25	0.51
3	22	25	0.50
4	22	25	0.50
5	22	25	0.49
6	22	25	0.46
7	22	25	0.51
		Av.	0.503

TABLE XIII.—BERRY AND CALYX OF ATROPA BELLADONNA[a]

Sample No.	Temp., ° C.	Wt., ° C.	Alkaloid, %
1	22	25	0.302
2	22	25	0.300
3	22	25	0.299
4	22	25	0.299
5	22	25	0.296
6	22	25	0.295
7	22	25	0.297
		Av.	0.298

TABLE XIV.—RHIZOME AND ROOT OF ATROPA ACUMINATA[a]

Sample No.	Temp., ° C.	Wt., Gm.	Alkaloid, %
1	20	20	0.526
2	20	20	0.532
3	20	20	0.521
4	20	20	0.525
5	20	20	0.526
6	20	20	0.527
		Av.	0.526

[a] All samples were dried by spreading out in a drying room with an average temperature of 22°. Drying time was from four to five weeks.

TABLE XV.—RHIZOME AND ROOT OF ATROPA BELLADONNA[a]

Sample No.	Temp., ° C.	Wt., Gm.	Alkaloid, %
1	20	20	0.505
2	20	20	0.515
3	20	20	0.508
4	20	20	0.510
5	20	20	0.510
6	20	20	0.510
7	20	20	0.510
		Av.	0.510

TABLE XVI.—STEM OF ATROPA ACUMINATA[a]

Sample No.	Temp., ° C.	Wt., Gm.	Alkaloid, %
1	20	10	0.208
2	20	10	0.196
3	20	10	0.201
4	20	10	0.203
5	20	10	0.199
		Av.	0.201

TABLE XVII.—STEM OF ATROPA BELLADONNA[a]

Sample No.	Temp., ° C.	Wt., Gm.	Alkaloid, %
1	20	10	0.195
2	20	10	0.192
3	20	10	0.190
4	20	10	0.195
5	20	10	0.192
		Av.	0.193

[a] The material used as samples in this series of assays consisted of the upper halves of the aerial stems.
Owing to the coarse, splintery nature of the ground drug only 10 Gm. could be placed in the extraction thimble.

TABLE XVIII.—LEAVES OF ATROPA ACUMINATA

Sample No.	Temp., ° C.	Wt., Gm.	Alkaloid, %
1	20	20	0.301
2	20	20	0.332
3	20	20	0.335
4	20	20	0.401
5	20	20	0.335
6	20	20	0.340
		Av.	0.342

TABLE XIX.—LEAVES OF ATROPA BELLADONNA

Sample No.	Temp., ° C.	Wt., Gm.	Alkaloid, %
1	20	20	0.299
2	20	20	0.325
3	20	20	0.324
4	20	20	0.328
5	20	20	0.324
6	20	20	0.328
7	20	20	0.325
8	20	20	0.320
		Av.	0.3216

All samples reported in Tables XIV–XIX were dried by spreading them out in a room with an average temperature of 20°. Drying time was approximately eight weeks.

Various portions of the plant were ground to a moderately coarse powder and extracted in a Soxhlet apparatus. The portion to be extracted was macerated overnight in a menstruum of 8 cc. of stronger ammonia T. S., 10 cc. of ethyl alcohol, and 20 cc. of ether. The following day the extraction was started, ether being added as the extraction menstruum. This extraction was allowed to continue for 24 hours.

The liquid, upon cooling, was transferred to a separatory funnel and the alkaloids were removed from the ether, using approximately half-normal sulfuric acid. Each portion was filtered as it was drawn off. The combined acid portions were rendered alkaline with ammonia T. S. and the alkaloids completely separated by extracting with successive portions of chloroform. The combined chloroform extractions were evaporated to dryness on a water bath and the heating continued for one-half hour. The residue was redissolved in chloroform and again evaporated in the above manner. This procedure was repeated for six successive times. The resulting residue was then dissolved in 4 cc. of chloroform, and 15 cc. of fiftieth-normal sulfuric acid was added. The chloroform was removed by evaporation on a water bath. The solution was allowed to cool, and the excess acid was titrated with fiftieth-normal sodium hydroxide, using methyl red T. S. as the indicator. Results were calculated as hyoscyamine using the U. S. P. XII (24) titration factor.

SUMMARY AND CONCLUSIONS

1. A brief history of the nomenclature is presented with the synonymy and the relevant literature.

2 The leaves, flowers, roots, and fruits of Indian Belladonna are described.

3. Histological studies have been made on Indian Belladonna. These studies include histological studies of the leaves, roots, and stems; vein islet number determinations; palisade ratios; and floral measurements.

4. Indian Belladonnda has an average palisade ratio of 8.3 and the European Belladonna an average palisade ratio of 6.1. The vein islet number for the Indian plant is 10.0. There is a variation in the stomatal number per square millimeter of leaf surface. The upper epidermis of the Indian variety showed an average stomatal number of 14.0 and the lower epidermis showed a stomatal number of 93.2. A similar investigation of the European variety yielded the following figures: upper epidermal count, 10.1, lower epidermal count 131.1. The stomatal index for the Indian variety is as follows: upper epidermis 3.4, lower epidermis 17.6. For the European variety: upper epidermis 2.9 and the lower 17.6.

5. The floral parts of the two varieties are similar in gross structure and insertion but differ mainly in the fact that the Indian variety exhibits larger organs and possesses a corolla which is bright yellow in color.

6. The root and stem histological studies revealed that basically the cellular structures are similar with the exception that the cells of the Indian variety are larger than those of the European.

7. The Indian variety possesses a higher alkaloidal content calculated as hyoscyamine.

8. Drying under infrared lamps at a very high temperature destroys the alkaloidal content.

9. No significant increase of the alkaloidal content was noted following the injection of certain amino acids into the plant tissues of the two varieties.

10. From the past discussion, we conclude that as yet we do not find sufficient variation in the two plants to warrant the present trend of establishing a separate species for the Indian variety. As a result, therefore, we advance the suggestion that, instead of referring to the Indian variety of Belladonna as *Atropa acuminata* Royle ex Lindley, it be called *Atropa Belladonna* L. variety *acuminata*.

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The Identification of Chinese Antimalarial Plants*

By S. T. YANG†

Five definitely identified plants indigenous to China which have been used as antimalarial drugs are briefly described. The present knowledge of these drugs is not complete, but that which is known is reported.

DURING the past five or six years a great deal of interest has been evinced in the study of antimalarial plants both in America and China—in America, because much of the fighting was carried on in the tropics where malaria was a very serious problem, and because, after the fall of Java, the largest source of quinine supply in the world was cut off from the Allies; in China, because the Chinese populace had retreated to the Southwest and established great military, economic, and cultural bastions in Szechuan and Yunnan provinces, the densest malarial region of that country. The southern portion of Yunnan being subtropical in climate and extremely mountainous, malaria, both benign and malignant, rages fiercely in that region. It is an established fact that in times past whole villages and towns were occasionally wiped out by this scourge.

The writer was one of the group of faculty and students connected with the three universities in North China who made the long trek to Kunming and established there the war-time National Southwest Associated University. Being an organic chemist, especially interested in natural and synthetic drugs, his attention was naturally directed to those antimalarial plants indigenous to that region. We also had the good fortune of having several competent botanists on our staff who were devoted students to the native flora. Consequently, in our nine years' stay (eight for the author, because he came to this country in 1945), we became well acquainted with about half a dozen of these plants. It might be of interest to those who are engaged in, or intend to undertake, the study and investigation of antimalarial plants to have available a list of names, describing their occurrence, giving the botanical identification, and summarizing briefly the present knowledge of these substances.

In Chinese books on materia medica, two plants

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are usually classified as antimalarials: *Ch'ang Shan* especially, and *Chai Hu*. For several reasons, their correct botanical identification, however, was not achieved until the last few years. In the first place, malaria is not prevalent in most parts of China, and the people do not have much use for these drugs. In the second place, these plants are found so far in the interior of China that many people have never had an occasion to see them. In the third place, herbs with antifebrile properties are often designated by local druggists as *Ch'ang Shan* or *Ch'ang* substitutes; thus, we have *Tien Ch'ang Shan*, *Tu Ch'ang Shan*, and a host of others. Therefore, a great deal of confusion was encountered in the identification of the original plants designated several thousand years ago by these names. Several plants besides *Ch'ang Shan* were also found to be used for the same purpose by people of different localities. In addition to *Chai Hu*, which has been reported to be *Bupleurum falcatum* Linn., and the two species of *Ch'ang Shan* investigated by the Japanese before World War II, *Orixa japonica* Thunb. (1) and *Hydrangea opuloids* Steud. (2), there are, at present, six plants known to be used in the treatment of malaria. Of the six, five are definitely identified as *Fraxinus malacophylla* Hensl., *Fraxinus chinensis* Roxbg., *Clerodendron yunansis* Hu, *Alstonia yunnansis* Diel, and *Dichroa febrifuga* Lour. It is still not quite settled whether the one found in Szechuan is white or *Pei Ch'ang Shan*.

CHINESE DRUGS

***Fraxinus malacophylla* Hensl.**—This plant is found in the southeastern part of Yunnan under the local name of *Pei Chiang Kan*. It grows in the limestone hills of that warm, humid, subtropical region around Wen Shan, Ma Kwan, and Kuang Nan. It is a tree of the ash family and attains a normal height of 20 to 30 feet in its native habitat. A specimen was brought to Kunming several years ago and has been kept alive on the grounds of a local hospital. The bark of the roots, in the form of a dark red powder with a bitter and astringent taste, is used by the local people as an antimalarial; this practice extends back for many centuries. Its use as an antimalarial was first reported by S. K. Liu (3) in 1941. Liu claimed to have isolated an alkaloid which had an antimalarial activity about ten times that of quinine. However, subsequent work by Jang (4) and Tonkin and Work (5) did not substantiate that claim, and no alkaloid was found. The nonalkaloidal constituent had no action at all on *Plasmodium gallinaceum* in chicks. This drug was also tested by the U. S. Government malaria testing station at Memphis, Tenn. (6), against *P. lophurae* in ducks, and the results were also negative.

***Fraxinus chinensis* Roxbg.**—This tree is found extensively in Szechuan and Yunnan, and, in fact, is one of the group used in the production of Chinese white wax or insect wax. It thrives best in moist places, along river banks or at the edge of a lake; hence, it is called *Sui* (water) *Pei La Shu* (white wax tree). It grows to a height of 4 or 5 to 10 or 12 feet. Originally, this plant was not used as an antimalarial by the Chinese people. As mentioned before, the tremendous migration of the Chinese people from the coastal provinces into the southwest interior, during our long war with Japan, brought arsenals and factories to Szechuan and Yunnan. The authorities were immediately confronted with the problem of malaria, and, because of the shortage of quinine and other modern antimalarial drugs, a fanatic search of native cures was started in 1941 and 1942. A Czechoslovakian medical doctor by the name of Schroeder, on the staff of the Applied Chemical Institute of the Chinese Ordnance Administration at Luchow, Szechuan, took note of this tree, because it was of the same family as the European ash, *Fraxinus excelsior* Linn. He told his colleagues that the bark of the European ash was used as an antimalarial by the people of the Balkans during the first World War, and it was probable that the bark of this Chinese tree might have the same property. Pressed by actual needs, and hearing that in Yunnan another tree of the same family was being used by the people there as an antimalarial, the institute authorities immediately began a study of this plant. Since the bark of *Fraxinus excelsior* had been investigated by Austrian chemists, who isolated a glucoside, Fraxin, and its aglucone, Fraxetin (7)—although no mention was made of its use as an antimalarial—Dr. K. H. Lih, the chemist-in-charge, and his colleagues extracted Fraxetin from the bark of the Chinese wax tree in 1942 (8). They made pills containing 10 per cent Fraxetin and conducted clinical experiments with malaria patients in the arsenals at Luchow. After two years they claimed that "Fenodin"—the name given to this tablet—was quite effective against both *P. vivax* and *P. falciparum*. Samples were sent to this country and Great Britain by Dr. Joseph Needham, of Cambridge University, England, who organized the Sino-British Science Coöperation Office in Chungking during the latter stages of the war. The tests on *P. lophurae* in ducks and *P. gallinaceum* in chicks were negative. Jang's results (4) also indicated that it was ineffective clinically, in the small number of cases tested.

***Clerodendron yunansis* Hu.**—This is a tree or tall shrub common in the environs of Kunming. It is very ornamental due to large masses of fine white flowers. Most Chinese books on materia medica describe two different kinds of *Ch'ang Shan:* one is *Yichow Ch'ang Shan* which has now been identified as *Dichroa febrifuga*, and the other, *Haichow Ch'ang Shan*, as *Clerodendron trichotomum*. Haichow was the old name for Kiangsu and Anhui. This Yunnan species is therefore frequently called *Tien Ch'ang Shan*. The majority of the drugstores in Kunming use thin slices of the twigs of this tree for prescriptions calling for *Ch'ang Shan*. On account of an unpleasant odor from its crushed leaves, it is also called by the local people *Hsiu* or foetid, *moutan shu*. So far as is known, no investigation has been conducted with this plant.

***Alstonia yunansis* Diel.**—This is a small shrub about 2 to 3 feet in height and grows wild in the fields of Kunming. Occasionally, it is planted in

gardens. Its profusion of small, bright red flowers and slender pea-like seed-pods is very picturesque. It is called *Chih Ku* or chicken bone *Ch'ang Shan* in Kunming (also in the book, "Identification of Chinese Plants, Drawings and Descriptions," Commercial Press, Shanghai), although this name is also used elsewhere to designate the best grade of *Dichroa* roots. The bark, twigs, and seed-pods are all used as the drug by an old and famous drug house in Kunming, Fu Ling Tang. It contains several alkaloids and resinous substances which show color changes in acid solution. It was found inactive against *P. lophurae* in ducks by the Memphis station (9).

***Dichroa febrifuga* Lour.**—This is an evergreen shrub found in the high tablelands of Szechuan and Yunnan. The main centers in Szechuan are Chungking, Hochuan, Ching-Fu Shan, and Wan Hsien; and in Yunnan, they are Yungping, Lungkai, Ch'angkai, and Tali. *Dichroa febrifuga* grows to a height varying from 4 or 5 to 9 feet. The leaves are opposite and lanceolate; and the flowers, on a pyramidal panicle, are numerous, small, and violet to blue in color, when fresh, and black when dry. The ripe fruit, about the size of a seed corn, contains many small seeds covered with fuzz. In Szechuan, only the roots are used; they are colored bright yellow. The use of the roots is strictly in accordance with the recommendations of Chinese books on materia medica. In fact, the name *Ch'ang Shan* is applied to roots only. The effectiveness of the leaves and young shoots was also noted in those classical books, but it was said *Shu Chi*, the name for the shoots and leaves, causes a stronger irritation to the stomach. In Yunnan, however, the dried leaves and sometimes the twigs are used, but never the roots. So, for quite a while we did not associate the Szechuan and Yunnan products to the same plant, knowing the loose use of the name *Ch'ang Shan* by local druggists.

This is the only antimalarial plant in this entire group, the effectiveness of which against avian and human malarias has been established beyond any doubt (3–6, 10). The leaves are found to be far superior to the roots in their effectiveness as an antimalarial.

It was not until the latter part of 1941 that we were able to obtain a small quantity of the powdered leaves. This drug was in the hands of a powerful local firm which wished to keep its source a trade secret from the public, especially the immigrants from outside. This company usually bought up most of the supply at its source, boiled the leaves with water into a thick black syrup, and then shipped the same to their Kunming factory for the manufacture of pills, under the trade name of "Chunine," and other galenical forms. Therefore, it was difficult to get any leaves as such in Kunming. Our first supply, from a friend, was quite small in quantity and finely powdered, so that we could not distinguish the original shape of the leaves. We learned, however, where the plants could be found. We had planned to send out a party to collect it in its native habitat, Yungping, in sufficient quantity for chemical studies, when, unfortunately, the Japanese Army invaded Burma in the beginning of 1942 and soon pushed into Yunnan. That portion of Yunnan on the western bank of the Salween River fell into enemy hands, and the region between Tali and the Salween River, where this plant is found, became the battle front. It was therefore impossible to send any party into that region for two years. The Japanese were finally mopped up in that section in the spring of 1944. That summer, with the help of the Chinese Army, an assistant was finally sent. After a difficult and hazardous journey, he visited all the three locales, but succeeded in procuring only a few pounds of the dried leaves and another few pounds of the twigs. He did bring back, however, two live specimens of the plant in question as well as some pressed specimens of the flowers and fruit. The reason for his meager success in securing a large amount of the leaves was that he had gone at the wrong time! The local drugstores usually keep about 25 to 30 pounds of the drug on hand, and seldom allow anybody to have more than a few ounces at a time, especially in view of the long malaria season with no fresh supply available until the next spring. Large quantities of the drug can only be had during the annual fair at Tali in the earlier part of April, or, more correctly, the middle of the third month according to the old Chinese calendar. The fair at Tali is quite a large affair; it is not only attended by merchants throughout Yunnan province but also by those from Szechuan, Hsi-Kang, Chinghai, Tibet, Burma, and India. Several thousand pounds of the leaves of *Dichroa febrifuga* can be purchased at that time. Thus, it was necessary to send another man the following spring to secure a sizable quantity. In the meantime, of course, we had identified the plant to be *Dichroa febrifuga*, identical with the Szechuan plant. The late Professor Y. T. Wu and Mr. Cho Po Chien, of Tsing Hua University, identified these different plants.

After identification, the most pressing problem was the isolation of the active principle or principles in pure chemical form. Jang in 1944 (4) claimed to have isolated two alkaloids from the root bark, but the work of several other investigators, including the writer (5, 6), has not confirmed his results. Some of us working on the root bark and others on the leaves have not been able to isolate any alkaloid thus far.

SUMMARY

There are six medicinal plants in China which may have antimalarial action; namely, *Fraxinus malacophylla* Hensl., *Fraxinus chinensis* Roxbg., *Clerodendron yunansis* Hu, *Alstonia yunansis* Diel, *Dichroa febrifuga* Lour., and an unidentified species.

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The Pharmacologic and Chemotherapeutic Action of *p,p'*-Diaminodiphenyltrichloroethane*

By M. I. SMITH, J. M. JUNGE, and W. T. McCLOSKY†

The toxicity, absorption, retention, metabolic fate, and chemotherapeutic action of *p,p'*,-diaminodiphenyltrichloroethane (DADT) were studied in comparison with the two analogs *p,p'*,-dichlorodiphenyltrichloroethane (DDT) and *p,p'*, - diaminodiphenylsulfone (DDS), and the results are reported.

IN A PREVIOUS study (1) of the pharmacologic action of a series of compounds chemically related to the insecticide DDT (*p,p'*-dichlorodiphenyltrichloroethane) it was shown that the characteristic neurotoxic and hepatotoxic actions of this substance are dependent on five halogens in the molecule. Compounds without the aromatic chlorine and compounds with one or more of the aliphatic chlorines removed had little or none of the actions of DDT on the central nervous system or the liver. Evidence of renal excretion of DDT and the chemically related chlorinated compounds or their degradation products was obtained by the demonstration of the presence of organic chlorine in the urine of rabbits treated with these compounds. Moreover, in the case of DDT it was shown that the excretory product in the urine of rabbits was the partially dechlorinated derivative *p,p'*-dichlorodiphenyl acetic acid (2, 3).

The present report concerns the pharmacologic properties and metabolic fate of another compound chemically related to DDT, a diamino analog of DDT, to be referred to for brevity as DADT. This compound, which was synthesized at the suggestion of one of us (M. I. S.) in the laboratories of the Geigy Chemical Co. and kindly supplied by Dr. V. Froelicher, seemed of particular interest for two reasons. First, it can be diazotized by the method of Bratton and Marshall (4) and therefore can be traced with relative ease and accuracy in the body tissues and fluids; secondly, it appeared to offer potential possibilities of chemotherapeutic activity because of its chemical relationship to *p,p'*-diaminodiphenylsulfone (DDS), the parent substance of many derivatives studied in this and in other laboratories for chemotherapeutic activity in experimental tuberculosis with a measure of success (5–8).

The structural relationship of this compound to DDT and DDS is shown below.

DDT $Cl-C_6H_4-\underset{\displaystyle C(Cl)_3}{\overset{\displaystyle H}{\underset{|}{\overset{|}{C}}}}-C_6H_4-Cl$

DADT $NH_2-C_6H_4-\underset{\displaystyle C(Cl)_3}{\overset{\displaystyle H}{\underset{|}{\overset{|}{C}}}}-C_6H_4-NH_2$

DDS $NH_2-C_6H_4-SO_2-C_6H_4-NH_2$

EXPERIMENTAL

The *acute toxicity* of DADT was studied in rats and mice. The base, being insoluble in water, was administered orally as an aqueous suspension with gum acacia or as a solution in oil.[1] It can also be administered orally or subcutaneously as the hydrochloride in 10% aqueous solution at a *p*H of 2.5–3.0, the substance being dissolved in a minimum volume of dilute HCl and made up to volume with H_2O. The results indicate that it appears to be absorbed best from oil-acetone solutions on oral administration.

Table I shows the results of the experiments on the acute toxicity of DADT and DDS in rats. The approximate LD_{50} of DADT in rats is between 1.0 and 1.5 Gm./Kg., and is therefore a little more than one-tenth as toxic as DDT, which is only 0.15 Gm./Kg. (9), thus following the general pattern of other DDT related compounds in which the aromatic chlorines are either lacking or replaced by other substituents (1). It is also somewhat less toxic than DDS in the rat.

TABLE I.—ACUTE TOXICITY IN RATS. ORAL ADMINISTRATION

Dose, Gm./Kg.	No. of Rats Used	No. Died	Per Cent Mortality
DADT 10% in corn oil			
2.0	3	3	100
1.5	10	8	80
1.0	20	4	20
DDS 10% aqueous suspension with gum acacia			
1.0	10	6	60
0.8	10	3	30

The systemic effects of DADT differ from those of DDT or DDS. Following the oral administration of DADT no symptoms are noticeable for an hour or two. Following this there is dyspnea, gen-

* Received April 9, 1948, from Experimental Biology and Medicine Institute, National Institute of Health, Bethesda, Md.

† Deceased January 12, 1948.

[1] It is rather sparingly soluble in oil, but readily soluble in acetone. A 10% solution of the substance in corn oil can be made conveniently by dissolving the substance in a minimum volume of acetone, 1 Gm. per cc., then adding the requisite amount of oil to volume.

eral muscular weakness, incoordination, ataxic gait, progressively difficult and labored respiration, coma, and death. Death may occur with large doses as 1.5 to 2.0 Gm./Kg. in two to five hours, or with smaller doses, as 1.0 Gm./Kg., in one to three days. There is no hyperirritability as in DDS poisoning, nor tremors and convulsions as in DDT poisoning.

The acute toxicity of DADT in mice is higher, as shown in Table II, than DDT, and somewhat lower than DDS. There is no adequate explanation at this time for the differences in susceptibility of the rat and mouse to these compounds.

TABLE II.—COMPARATIVE ACUTE TOXICITY IN MICE

Compound	Administration	Gm. per Kg.	No. Used	Per Cent Mortality
DDT	Oral in peanut oil	0.8	10	100
		0.6	10	100
		0.4	10	40
DADT	Oral in corn oil	1.0	10	100
		0.5	10	90
		0.25	10	40
DDS	Oral in gum acacia	0.5	10	60
		0.25	10	70
		0.10	10	0

linear curve, shown in Fig. 1, obtained by plotting absorption against concentration.

Blood levels in rats and guinea pigs, from single doses of 1.0 and 0.5 Gm./Kg., respectively, were low, of the order of 0.5 to 1.5 mg. per cent throughout the twenty-four-hour period following the administration of this compound. This probably is not due to poor absorbability since rats receiving 1.0 Gm./Kg. usually showed definite symptoms of intoxication, sometimes persisting for twenty-four to forty-eight hours. More likely it is a matter of distribution of the substance in the body favoring certain tissues in preference to the blood, but this has not been determined definitely.

In rabbits the blood levels varied in different animals (Table III). Usually blood levels of 2 to 8 mg. per cent were attained within a few hours following a single dose and this was maintained for several days. The excretion in the urine is greatest during the first and second days, but it continues for many days thereafter. Measurable amounts can be detected in the urine for ten to twenty days. The persistence of the substance in the urine for many days is strikingly analogous to DDT, for as has been shown previously organic chlorine, signifying DDT or degradation products, may persist in the urine of rabbits for ten days or longer following a single dose of DDT (10). On the other hand, the blood levels of DADT in rabbits resemble more nearly those of DDS (6), the blood levels of DDT in rabbits being

TABLE III.—BLOOD LEVELS AND URINARY EXCRETION OF DADT IN FOUR RABBITS, WEIGHING 2–2.5 KG ORAL ADMINISTRATION OF 0.5 GM./KG., 10% SOLUTION IN CORN OIL

Time	Blood Levels, Mg. %				Urinary Excretion, Mg. Total			
	1	2	3	4	1	2	3	4
2–4 Hr.	7.0	2.0	2.2	6.1	..	...	...	...
24 Hr.	8.5	2.0	6.0	8.0	92	156	163	144
48 Hr.	...	0.8	4.5	4.2	12	50	69	196
72 Hr.	...	1.0	3.7	1.0	12	12	11	68
96 Hr.	3.0	0.4	1.0	...	12	2	12	22
6 days	1.0	...	...	...	5	9	2	11
7–8 days	0.8	0.4	0.3	0	6	10	...	...

The acute toxicity in other animal species has not been studied thoroughly, but twelve guinea pigs and eight rabbits given single doses of 0.5 Gm./Kg. in corn oil by stomach tube for metabolic studies survived and showed no visible toxic effects. If it is remembered that rabbits almost invariably show definite tremors from 0.05 Gm./Kg. DDT (9) and that the LD_{50} of DDT for rats is 0.15 Gm./Kg., while guinea pigs usually succumb to 0.25 Gm./Kg. DDS (6), it would appear that DADT is less toxic than the other two compounds in both species.

Metabolism.—The metabolism of DADT was studied in rats, guinea pigs, and most thoroughly in rabbits. The concentration of the substance in the blood and urine was studied by a modified Bratton and Marshall (4) technique. Ten to fifteen minutes are required for the complete formation of chromogen on diazotization and coupling and during this time much of the chromogen forms colloidal aggregates, as in the case with many sulfones. For this reason 5 cc. of acetone was added in all cases just before reading to redissolve the chromogen. The Fisher Electrophotometer was used with B 525 filter, and the light absorption recorded. From these data the concentration of DADT was computed from the standard

usually of a very low order (10). Thus it would seem that DADT stands as an intermediary between DDT and DDS, having the characteristics of the former in so far as persistence of action is concerned, with absorption and distribution qualities of the latter.

In our studies on the metabolic fate of DADT advantage was taken of its physicochemical characteristics, *viz.*, the insolubility of the base in water and its solubility in organic solvents, and the solubility of the hydrochloride in water and its insolubility in ether. It was noted that the urine of rabbits, which is normally alkaline, was strongly opalescent, almost milky in appearance for two to three days following the administration of a single dose of DADT. This completely cleared when shaken with ether or when strongly acidified with HCl. The following procedure was therefore adopted for the isolation of the substance from the urine.

The urine was alkalinized with dilute NaOH solution to *p*H 10–11, shaken out two or three times with ether, the ether and emulsion layer if any dehydrated with anhydrous Na_2SO_4, and washed once or twice with water. The washed ether was then extracted thoroughly by shaking in a separatory funnel two or three times with dilute HCl in H_2O

at *p*H 2.0 or less. The acid aqueous extract was then alkalinized with NaOH to complete precipitation and this extracted with two or three portions of ether. This procedure can be repeated several times without appreciable loss of material until the acid aqueous extract is almost colorless. The last traces of pigment were removed by shaking with a small amount of charcoal (Nuchar W) and filtered. The colorless filtrate was then alkalinized with sufficient NaOH solution to complete precipitation, the precipitate centrifuged off and washed with water by centrifugation. The packed precipitate was dissolved in a few cc. of hot ethyl alcohol in which it is very soluble. The alcoholic solution was allowed to cool, and any insoluble material was removed after centrifugation. To the decanted clear alcoholic solution 2 volumes of water was added cautiously until slight turbidity began to appear. On standing for several hours, with frequent rubbing of the sides of the centrifuge tube with a glass rod, crystallization occurred and this was allowed to proceed until the supernatant liquid was clear and remained clear on further addition of small amounts of water. When crystallization was complete the material was centrifuged off, the supernatant liquid decanted, and the centrifugate dried *in vacuo*.

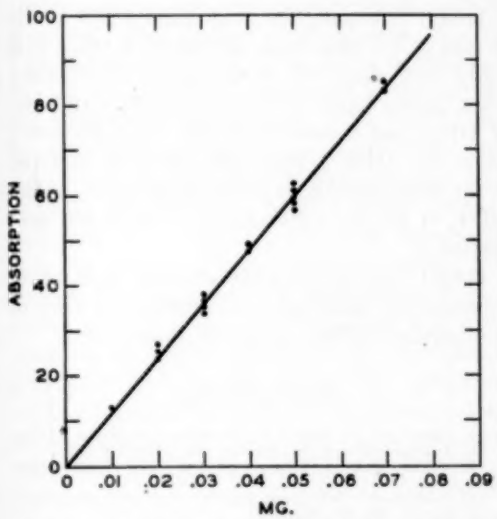

Fig. 1.—Absorption-concentration curve of DADT as measured by the Fisher Electrophotometer with B 525 filter. Ordinates show light absorption; abscissae-concentration in mg. per 18 cc., including 5 cc. acetone.

Good yields were thus obtained, in relation to the material originally present in the urine based on determinations by the diazotization technique. This crystalline material had a sharp melting point of 146–8°.[2] A solution of this gave upon diazotization and coupling an absorption-concentration curve quantitatively identical with that of the compound itself. Moreover, DADT and the substance isolated from the urine, when nitrated in equivalent amounts and a benzol solution of the nitrated compounds treated with sodium methylate in methanol by the method of Schechter and Haller (11), gave identical absorption curves within the range of 460–700 mμ, with a maximum absorption in both cases at 580–600 mμ, as shown in Fig. 2.

Microanalyses,[3] of a sample of DADT and two samples isolated from the urine of rabbits gave the following results.

	Carbon	Hydrogen	Chlorine
DADT	53.41	4.22	32.54
Urine sample one	53.73	4.73	32.60
Urine sample two	53.54	4.66	32.04
Theory	53.27	4.15	33.10

These results indicate that the material extracted from the alkalinized rabbit urine is probably unaltered DADT. It is probable that DDS is excreted in a similar way, though accurate information on this is not available. The compound DDT, on the other hand, it has been abundantly demonstrated (2, 3), is excreted in the urine of rabbits as *p,p'*-dichlorodiphenyl acetic acid. In this respect, therefore, DADT resembles DDS more closely than DDT.

Chemotherapeutic Activity.—The chemotherapeutic activity of DADT was examined in experimental pneumococcus infection (Type I) in mice, and in experimental tuberculosis in guinea pigs. The antipneumococcal tests in mice were carried out in the usual manner (12). The mice were inoculated intraperitoneally with 0.5 cc. of 10^{-6} dilution of a six-hour veal infusion blood broth culture, which kills all controls in twenty-four to forty-eight hours, and the drug was administered for four days twice daily in doses of 20 to 50 mg./Kg. The individual doses were from 8 to 20% of the LD_{50}. For comparison groups of infected mice were also treated with equivalent doses of DDT and 75 mg./Kg. of DDS. The results shown in Table IV indicate no chemotherapeutic activity from DADT or DDT, with a fair degree of chemotherapeutic activity from DDS, as has usually been seen in this laboratory in routine testing of sulfone derivatives, with DDS being used as a standard of reference (12).

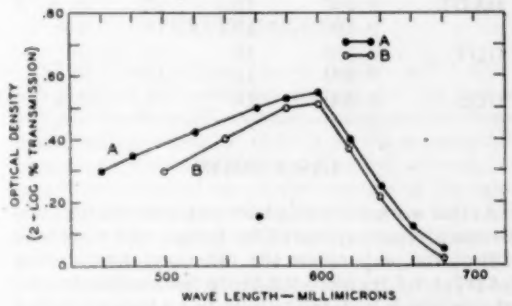

Fig. 2.—Absorption curves of nitrated DADT in 5 cc. benzol + 10 cc. of 10% sodium methylate in methanol. Bausch & Lomb universal spectrophotometer. All measurements made at a depth of 1.5 cm.

A. 2 mg. DADT. *B*. 1.8 mg. of material isolated from rabbits' urine.

In the test for chemotherapeutic activity in experimental tuberculosis twenty male guinea pigs of

[2] The melting point of the DADT used was 153–4°. Mixed melting point 148–9.

[3] For this we are indebted to C. A. Kinser of this Institute.

about 300 Gm. each were inoculated intraperitoneally with 0.5 mg. tubercle bacilli human strain suspended in 1 cc. sterile salt solution. One group of 10 was kept as controls and the animals of the second group were treated daily with 0.25 Gm./Kg. DADT for the first two weeks, and 0.10 Gm./Kg. for the following eight weeks. The dose was reduced on account of evidence of toxicity, probably due to cumulative action. All but two of the controls died in forty-seven to ninety days, and all of the treated animals died in seventeen to ninety days. The two surviving controls were then killed and the experiment terminated. At autopsy the extent of tuberculous involvement was noted and rated as previously described (6). The T. B. index of the ten controls varied from 4 to 16 with an average of 10.7 out of a possible maximum of 20. In the group of treated animals the range was 7 to 18 with an average of 12.2. In a group of 10 animals similarly infected and treated concurrently with 10 mg./Kg. streptomycin twice daily intramuscularly all survived and when killed and autopsied the extent of tuberculous involvement ranged from 1 to 4 with an average T. B. index of 1.5. Obviously DADT had no therapeutic effect in tuberculosis under the experimental conditions.

TABLE IV.—THERAPEUTIC TEST. PNEUMOCOCCUS (TYPE I) INFECTION IN MICE

Drug	Total Dose, Gm./Kg. per os	No. of Mice	No. Died	Survival, %
DADT	0.160	15	15	0
	0.400	15	14	6.6
DDT	0.160	15	15	0
	0.400	15	15	0
DDS	0.600	14	8	42.8

DISCUSSION

As this work was well under way and nearing completion a report appeared by Burger and associates (13) on the preparation of a dibenzoyl derivative of DADT, 1,1,1-trichloro-2,2-bis-(*p*-benzamidophenyl)-ethane, which is stated to have had a high inhibiting action *in vitro* against the tubercle bacillus. They were apparently unable to get the free base. Shortly afterward Kirkwood and associates (14) reported on the synthesis of DADT with a melting point of 92–95°, which inhibited the growth of the tubercle bacillus *in vitro* in a concentration of 1:100,000 and *in vivo* tests "showed remarkable control of experimentally induced tuberculosis in guinea pigs." In a later publication Kirkwood and Phillips (15) reported the preparation of DADT with a melting point of 154–55° with a tuberculostatic activity *in vitro* of 1:100,000 and with indications of possible activity *in vivo*, but with no details. Our compound had a melting point of 153–54°. It was so insoluble in the usual liquid media at *p*H 7.0 that *in vitro* tests for tuberculostatic activity were not considered feasible.

It may be of interest to comment briefly on the relation of chemical constitution to pharmacologic and chemotherapeutic action as exemplified by the three compounds in this study. The toxicity of DADT in some species as the rat and rabbit is less than that of DDT and is consistent with previous findings which indicated that dechlorination of the DDT molecule resulted in compounds of lower toxicity. The diamino substituted derivative is also free of neurotoxic action so characteristic of DDT. The substitution of two amino groups in place of the two aromatic chlorines in DDT has had the effect of giving much higher blood levels, and of blocking the metabolic degradation of the trichloroethane bridge to the acetic acid derivative. Comparing DADT with DDS it is evident that the trichloroethane bridge makes for lower toxicity and much longer retention in the body, while all chemotherapeutic activity possessed by the sulfone appears to have been completely eliminated by the substitution of the trichloroethane group for the SO_2 group.

SUMMARY AND CONCLUSIONS

The toxicity, absorption, retention, metabolic fate, and chemotherapeutic action of *p,p'*-diaminodiphenyltrichloroethane (DADT) were studied in comparison with the two analogs *p,p'*-dichlorodiphenyltrichloroethane (DDT) and *p,p'*-diaminodiphenylsulfone (DDS).

DADT is usually less toxic than DDT and is devoid of the neurotoxic actions of DDT. It is fairly well absorbed from the gastrointestinal tract, giving blood levels comparable with those of DDS, and is retained in the body for many days, like DDT. That part which is excreted in the urine appears to be unchanged and, unlike DDT, it is not metabolized to an acetic acid derivative.

DADT has no chemotherapeutic activity in experimental pneumococcus infection in mice or in experimental tuberculosis in guinea pigs.

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An Investigation of the Assay of Ascaridol. II. The Iodination of Terpenes*

By ALFRED HALPERN†

The rate of iodine released in the assay of ascaridol was determined. The major portion of the iodine was released within the first minute of the reaction. A slower steady release of iodine was also evidenced. The possibility of iodinating unsaturated hydrocarbons structurally related to ascaridol was investigated. The olefinic linkage in these compounds was not iodinated under the conditions of the official assay for ascaridol. The nonstoichiometric behavior of ascaridol toward the iodide ion cannot be explained by the simple iodination of olefins.

In an earlier investigation of the assay of ascaridol in oil of chenopodium, some of the difficulties involved in the determination were reported (1). The method that had the fewest limitations was the procedure of Cocking and Hymas (2) involving the liberation of iodine from acidified potassium iodide by the organic peroxide ascaridol. This method is official in N. F. VIII.

One of the chief criticisms of this method was the use of an empirical equivalent in the calculation of the ascaridol content. The reaction between the ascaridol and the potassium iodide does not proceed in the expected stoichiometric relationship, but one mole of iodine is equivalent to 0.66 mole of ascaridol. This was determined from the mean of a series of experiments. It is important to note that this relationship was reproducible. The authors (2) claimed that three reactions occurred in sequence: the liberation of 2 moles of iodine from the acidified potassium iodide solution, followed by an unexplained liberation of iodine, and then when the solution was diluted, an absorption of iodine took place.

The iodometric determination of peroxides is a rather well-known procedure (3–5). Most of these determinations are similar to the one proposed by Cocking and Hymas. The estimation of peroxides by these techniques was quantitative for many peroxides. However, difficulties were noted in the case of others (e.g., cyclohexene-peroxide).

In the ascaridol assay the absorption of iodine might be explained by the addition of iodine to an unsaturated olefin. Ascaridol has an unsaturated linkage in its molecule. The diiodide, if formed, might also be reduced by the hydrogen iodide present, to liberate free iodine, thus accounting for the nonstoichiometric quantity of iodine present. Another explanation would be the reaction between the decomposition products of ascaridol and hydrogen iodide to liberate free iodine.

The official determination of ascaridol content is carried out in the presence of the iodide ion. Iodine, in the presence of the iodide ion, forms the triiodide ion. The triiodide ion has been shown to inhibit the iodination across the unsaturated linkage (6). The presence of ascaridol, a peroxide, might catalyze this addition.

The present study was primarily concerned with the behavior of unsaturated olefins related to ascaridol under the experimental conditions of the assay. The possibility of iodinating these compounds and the influence of these iodides, if formed, on the quantity of iodine liberated during the course of the reaction was investigated.

EXPERIMENTAL

Oil of Chenopodium (d_{25} = 0.959; n_D^{25} 1.4768) was assayed for ascaridol content by the iodine method (N. F. VIII) and the average of five determinations corresponded to 69% ± 4% of ascaridol. In order to determine the rate of iodine released, the assay was repeated on another sample of the same oil. After the indicated lapse of time a 1-ml. aliquot was withdrawn and the iodine content immediately determined by titration with hundredth-normal sodium thiosulfate solution. The amount of iodine liberated and the equivalent ascaridol content were determined (Table I and Fig. 1).

TABLE I.—IODINE LIBERATION DURING THE ASSAY OF ASCARIDOL

Time, Min.	Iodine Mg.[a]	Ascaridol Equivalent, %[b]	
1	13.1	59.8	
2	14.1	62.1	
3	15.5	66.3	
4	15.6	66.4	End of N. F. VIII
5	16.0	69.8	time period
7	16.6	73.2	
10	16.9	74.5	
15	17.0	74.8	
20	17.1	75.1	

[a] Present in a 1-ml. aliquot. The indicated quantities represent the mean of three determinations.
[b] Based on a 0.2750-Gm. sample in 23 cc. of reaction medium.

The results of the iodine-release study indicated that the greatest amount of iodine was liberated

* Received February 17, 1948, from the School of Pharmacy, Duquesne University, Pittsburgh, Pa.
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within the first minute of the reaction period. There was a steady release of iodine throughout the entire period of observation (twenty minutes). Between zero and one minute 13.1 mg. of iodine was liberated, corresponding to 59.8% of ascaridol (based on a 0.275-Gm. sample) and during the nineteen-minute period following an additional 4 mg. of iodine was liberated, corresponding to 15.3% of ascaridol. There was no observable absorption of iodine during this period. This was in agreement with the observation of Cocking and Hymas (2) that there was an immediate liberation of iodine followed by a second unexplained liberation of iodine.

The cyclic peroxides of the 1,4 type and their conjugated polymers have been shown to behave differently toward the ferrous and iodide ions (6, 7). The reaction proceeds much more slowly with the bridge-type peroxides than with the simple dihydroperoxides. Ascaridol, a monomeric 1,4 bridge-type cyclic peroxide, might be expected to react at a slower rate and the results in this case would be empirical. The rate study, however, implies that two reactions take place and that the first reaction proceeds at a much greater rate than the second one. We hesitate to draw conclusions from a limited amount of data and a more intensive study of the kinetics of the reaction is now in progress. These results will be published at a later date.

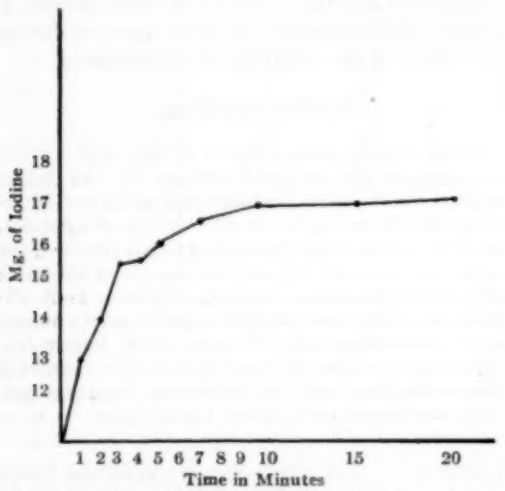

Fig. 1.—Iodine Liberation During the Assay of Ascaridol.

Ascaridol has been considered structurally and chemically similar to the auto-oxidation product of 1,3 menthadiene (7). Presumably when the oxygen is given up an olefinic compound results. This would be expected from analogy to related compounds, such as rubrene peroxide which forms rubrene on decomposition (8). The formation of olefins might account for the absorption of iodine reported by Cocking and Hymas (2). To investigate adequately the role of the unsaturated compounds in the possible absorption and latent liberation of iodine, a series of olefins related to ascaridol were studied in the light of their tendency to be iodinated under the condition of the assay.

Preparation of *p*-Menthene.—*p*-Menthene was prepared by the dehydration of menthol with copper sulfate in a manner similar to the one described by Richtman and Kremers (9). Presumably, the resultant menthene was a mixture of Δ^2-menthene and Δ^3-menthene. Since it was not important for the purposes of this investigation to obtain the pure individual isomer the mixture was distilled and the fraction boiling at 171–174°/760 mm. was collected. It had the following physical properties: $d_{20} = 0.826$, $n_D^{20} = 1.4601$.

The nitrosochloride was prepared in the usual manner and melted at 90–91°.

Preparation of Terpinolene.—In a 1-liter, 3-neck flask fitted with a dropping funnel and an inlet tube for steam and an exit tube connected to a condenser, was placed 500 ml. of a saturated oxalic acid solution This solution was heated to boiling and a mixture of 30.8 Gm. (0.2 mole) of terpineol in 100 ml. of hot (80–90°) water was added dropwise. A current of steam was passed through the reaction medium to remove the terpinolene as soon as it was formed. When all of the terpineol was added, the steam distillation was continued for another fifteen minutes. The distillate was extracted with three 50-ml. portions of petroleum ether and dried over anhydrous sodium sulfate. The solvent was removed under diminished pressure and the residue distilled. The fraction boiling at 67–68° at 10 mm. of mercury pressure was collected. The micro boiling point at atmospheric pressure was 184–185°. Yield: 56% of theory. The tetrabromide was prepared and it melted at 116–117°.

d-Limonene, dipentene and *p*-cymene were obtained commercially and redistilled. The fraction boiling over the described range (Table II) was collected and the physical properties determined.

IODINATION STUDY

I. Iodination Study

Approximately 1 mM of the appropriate unsaturated compound, accurately weighed, in 5 cc. of 90% acetic acid, was added to a 125-ml. Erlenmeyer flask containing a solution of 5 mM. of iodine in 5 ml. of hydrochloric acid and 10 ml. of glacial acetic acid, previously cooled to −3°. The flask was stoppered and allowed to stand in a cool place for ten minutes. The temperature was maintained at 0–5°. The contents were then titrated with 0.1 *N* sodium thiosulfate solution. The amount of iodine absorbed by the experimental olefins was determined (Table III).

II. Iodination of the Presence of the Iodide Ion and the Triiodide Ion

This procedure was the same as described above (I) except that 3 cc. of an 83% (*w/v*) potassium iodide solution was added to the iodine-HCl-acetic acid mixture prior to the addition of the unsaturated compound. The iodine content was determined at the end of ten minutes by titration with 0.1 *N* sodium thiosulfate solution (Table III).

The unsaturated compounds all absorbed appreciable quantities of iodine, with the exception of *p*-cymene which was a benzene derivative and consequently would not be expected to iodinate readily under the conditions of the experiment. Iodination in the presence of the iodide ion, however, resulted in

an almost quantitative recovery of iodine, indicating no iodination of the olefinic linkage. The triiodide ion has been shown to depress the iodination of unsaturated compounds. Wagner, Smith, and Peters (6) have studied the iodination of cyclohexene and isoprene in the presence of the triiodide ion, as a phase of an investigation of the determination of peroxides and have noted similar results.

SUMMARY

1. The rate of iodine released in the assay of ascaridol was determined. The major portion of the iodine was released within the first minute of the reaction. A slower steady release of iodine was also evidenced.

TABLE II.—PHYSICAL PROPERTIES OF TERPENES

Compound	760 Mm.	Specific Gravity, d^{25}	Refractive Index, n_D^{20}	Source
Menthene	171–174°	0.826[a]	1.4601	Synthetic from menthol
d-Limonene	174–176°	0.849	1.4749	Eastman Kodak
Dipentene	175–176°	...	....	Eastman Kodak
Terpinolene	184–185°	0.854	1.4840	Synthetic from terpineol
p-Cymene	176.5–177.5°	0.860	....	Eastman Kodak

[a] d^{20}.

TABLE III.—THE IODINATION OF TERPENES

Compound	Millimoles Present	Method I: Millimoles of I_2 Added[a]	Method I: Millimoles of I_2 Found	Method II: Millimoles of Iodine Present as I_2[b]	Method II: Millimoles of I_2 Found
Menthene	1.12	5.0	4.1	5.0	4.98
Terpinolene	1.41	5.0	2.8	5.0	4.99
d-Limonene	1.61	5.0	2.6	5.0	4.99
Dipentene	1.53	5.0	2.9	5.0	4.98
p-Cymene	1.43	5.0	4.5	5.0	5.00

[a] In 5 cc. of glacial acetic acid.
[b] Iodine added to the KI solution so that 3 cc. of solution contains 5 mM of I_2.

To establish the possible effect that peroxides might have on the iodination of the unsaturated compound, the reaction was repeated in the manner described for Method II and 0.01 mole of ascaridol was added with the olefin. A blank was run at the same time without the olefin. The iodine liberation due to the ascaridol was determined and subtracted from the experimental value. The results indicated that there was no absorption of iodine and were identical with Method II. This conclusively established that the olefins were not iodinated under the conditions of the experiment.

Since it was established that the iodination of the olefinic linkage did not occur under the conditions of the assay, the possibility was nonexistent for the iodides to become a source for the unexplained secondary liberation of iodine. For this reason the experimental determination of the stability of these iodides in the presence of hydrogen iodide and ascaridol was not carried out at this time. However, it was of interest to note that a simple alkyl iodide (ethyl iodide) was reported (6) to be perfectly stable under conditions analogous to those of the N. F. VIII for the assay of ascaridol.

It was evident from the above results that an explanation for the atypical behavior of ascaridol toward the iodide reagent was not the simple iodination of the olefinic linkage.

2. The possibility of iodinating unsaturated hydrocarbons structurally related to ascaridol was investigated. The preparation of menthene and terpinolene was described.

3. The olefinic linkage in these compounds was not iodinated in the presence of the triiodide ion.

4. The nonstoichiometric behavior of ascaridol toward the iodide ion cannot be explained by the simple iodination of olefins.

5. Further work is now in progress on an investigation of the kinetics of the reaction and the intermediates formed in the course of the determination.

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A Study of the U. S. P. XIII Method for the Assay of Desiccated Thyroid*

By HAROLD C. HEIM

A reagent blank titration and a "slipping" end point are frequently encountered in the U. S. P. XIII method for the determination of iodine in desiccated thyroid. The reason for this condition appears to be due to the addition of bromine to a warm alkaline solution with the probable formation of oxidized bromine compounds which in turn liberate iodine in the blank. By modifying the method so that the filtrate is acidified before adding the bromine, neither "slipping" end points nor reagent blank titrations were encountered, and recoveries of iodine in close agreement with theoretical values were obtained. Titrations were conducted at various *p*H values ranging from 1.2 to 3.2.

SEVERAL investigators have noted the returning or "slipping" end point in the various alkaline fusion methods for the determination of iodine in desiccated thyroid. Johnson and Nelson (1) recommend that the final titration be conducted between *p*H limits of 2.2 and 2.5. They state that at *p*H values below 2.0, the rate of return of color increases. Waters and Beal (2) conclude that the return of color results from the use of an excess of bromine, with the possible formation of an oxidized bromine compound which does not dissociate at the *p*H of the titration. These authors also note an increased intensity of color in the reagent blank. They found that if the bromine was added at *p*H values below 4.5, the end point did not fade, but that when the bromine was added to solutions at this *p*H, low results were obtained. They state this condition was caused either by incomplete oxidation or by loss of iodine. Block and Waters (3) report that, regardless of the normality of the thiosulfate solution used, the bromine should be purified before further use if a reagent blank greater than 0.2 cc. is obtained. Karns and Donaldson (4) describe a method for purifying the bromine used in the determination of iodine.

The Methods of Analysis of the Association of Official Agricultural Chemists include the Elmslie-Caldwell method for the determination of iodine (5). This method is, like the U. S. P. XIII method, an alkaline fusion method but differs from the U. S. P. method in that the bromine is added after the filtrate is acidified. With this method slipping end points and reagent blanks are rarely, if ever, encountered. Johnson and Frederick (6) studied the Elmslie-Caldwell method and reported low results when the method was applied to mineral mixtures containing little or no organic matter. When organic matter was added, however, these authors obtained better recoveries of the added iodine.

The present study was undertaken in order to compare the accuracy of the U. S. P. XIII and Elmslie-Caldwell methods and to determine the effect of modifying the U. S. P. XIII method by adding the bromine after the filtrate had been acidified.

EXPERIMENTAL

A mixture composed of casein, starch, and recrystallized *o*-iodobenzoic acid (m. p. 162°) was assayed by the U. S. P. XIII method, by the Elmslie-Caldwell method, and by the U. S. P. XIII method modified by acidifying the filtrate before adding the bromine. Another series of assays, using the same methods, was run on desiccated thryoid powder and on aliquots of a solution composed of reagent grade potassium iodide in distilled water. The *p*H was determined with a glass electrode apparatus prior to the addition of the potassium iodide and starch indicator. All titrations were carried out at 25° ± 1° with $N/200$ thiosulfate. The ignitions were conducted by heating for thirty minutes at 600°, and reagent grade chemicals were used throughout. The bromine was labeled to contain not more than 0.05% iodine and was purified before use according to the method of Karns and Donaldson (4). The results of these experiments are set forth in Tables I, II, and III.

In order to determine the effect of *p*H on the reagent blank, a series of blanks was run at *p*H values ranging from 1.2 to 3.2. The quantities of reagents used were those directed by the U. S. P. XIII with the exception that the phosphoric acid was added until the desired *p*H was obtained. One-half of the blanks were run as directed by the U. S. P. XIII method and in the other half of the blanks, the acid was added before the addition of the bromine. These results are given in Table IV.

DISCUSSION

The results shown in Tables I and II indicate recoveries of iodine in close agreement with the theoretical values. Low results were not obtained when the filtrates were acidified before adding the bromine, although Waters and Beal (2) have reported results as much as 25% low when they carried out the oxidation in this manner. Furthermore, in none of the assays here reported was a reagent blank obtained when the bromine was

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added to the acidified filtrate even though titrations were carried out at *p*H values ranging from 1.2 to 3.2. With the U. S. P. XIII method, however, slowly developing color in the reagent blanks was noted in every instance, and "slipping" end points were frequently encountered. Table III indicates that comparable results are obtained by all three methods in the assay of desiccated thyroid and also shows the slow development of color in the reagent blank in the U. S. P. XIII method.

TABLE I.—RECOVERY OF IODINE FROM POTASSIUM IODIDE SOLUTION

Method	Gm. I Found	Gm. I Added	*p*H	Cc. Blank	Remarks
U. S. P. XIII	0.00186	0.00186	2.2	0.1	Color developed slowly in blank.
	0.00244	0.00248	2.2	0.2	End point returned slowly
	0.00310	0.00296	2.3	0.1	
Modified U. S. P. XIII	0.00217	0.00217	2.2	0.0	No color in blanks after 10 minutes'
	0.00244	0.00248	2.3	0.0	standing. End point did not return
	0.00309	0.00310	2.4	0.0	
Elmslie-Caldwell	0.00245	0.00249	2.8	0.0	No color in blanks after 10 minutes'
	0.00305	0.00310	2.7	0.0	standing. End point did not return
	0.00279	0.00280	2.7	0.0	

TABLE II.—RECOVERY OF IODINE FROM MIXTURE OF *o*-IODOBENZOIC ACID, STARCH AND CASEIN

Iodine Content 0.0348%

Method	I Found, %	*p*H	Cc. Blank	Remarks
U. S. P. XIII	0.0338	2.3	0.015 (*p*H 2.2)	Color developed slowly in blank.
	0.0341	2.2		End point returned slowly
	0.0340	2.2		
Modified U. S. P. XIII	0.0341	2.3	0.0 (*p*H 2.2)	No color in blank after 10 minutes'
	0.0339	2.3		standing. End point did not return
	0.0347	2.2		
Elmslie-Caldwell	0.0348	1.6	0.0 (*p*H 2.6)	No color in blank after 10 minutes'
	0.0342	3.1		standing. End point did not return
	0.0338	2.9		

TABLE III.—ASSAY OF DESICCATED THYROID POWDER

Method	I Found, %	*p*H	Cc. Blank	Remarks
U. S. P. XIII	0.224	2.1	0.1 (*p*H 2.2)	Color developed slowly in blank.
	0.224	2.1		End point returned slowly
	0.220	2.2		
	0.225	2.3		
Modified U. S. P. XIII	0.223	2.1	0.0 (*p*H 2.8)	No color in blank after 10 minutes'
	0.224	2.2		standing. End point did not return
	0.220	1.4		
	0.226	3.0		
Elmslie-Caldwell	0.225	2.7	0.0 (*p*H 2.8)	No color in blank after 10 minutes'
	0.224	2.6		standing. End point did not return
	0.222	2.8		
	0.229	2.6		

TABLE IV.—EFFECT OF *p*H ON REAGENT BLANKS

Method	*p*H	Cc. *N*/200 $Na_2S_2O_3$	Remarks
U. S. P. XIII	1.2	0.1	Color developed within one minute of the
	1.5	0.1	time the potassium iodide and starch
	1.8	0.2	were added
	2.0	0.1	
	2.5	0.2	
	3.0	0.1	
	3.2	0.2	
Modified U. S. P. XIII	1.2	0.0	No color developed in this series of blanks
	1.5	0.0	even after 10 minutes' standing
	1.8	0.0	
	2.0	0.0	
	2.5	0.0	
	3.0	0.0	
	3.2	0.0	

In view of the fact that bromine, when added to warm, alkaline solutions, forms hypobromites, bromates, etc., it would appear possible that residual traces of such oxidized bromine compounds could cause the liberation of iodine in the reagent blank. If, however, bromine is added to an acid solution, these oxidized compounds of bromine are not formed.

Baumann and Metzger (7) report that alkali iodides are quite volatile, and that on heating iodides with alkali carbonates at temperatures as low as 85°, serious losses occur. The results shown in Table I do not corroborate their findings.

CONCLUSIONS

1. With the U. S. P. XIII method for the determination of iodine in desiccated thyroid, results very close to the theoretical values are obtained when the alkaline filtrate is made acid before adding the bromine.

2. The proposed modification of the U. S. P. XIII method eliminates the blank titration and the "slipping" end point, provided reagent grade chemicals are used throughout the determination.

3. "Slipping" end points and development of color in the reagent blanks were not observed with the modified method between *p*H limits of 1.2 and 3.2.

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Electrodialysis in the Field of Antibiotics*

By HENRY FISCHBACH†

THE INTENT of this report is to acquaint other investigators in the field with the value of a well-known technique, electrodialysis, hitherto not used in the study of Streptomycin or Bacitracin. It is known that both Streptomycin salt (1) and Bacitracin (2) will dialyze through a semipermeable membrane. In this laboratory it has been found that both of these antibiotics will migrate toward the cathode when the respective solutions are subjected to a direct current. Electrodialysis incorporates both of the above properties.

During the current investigations there has been a need for concentrating "pooled" mixtures of Streptomycin hydrochlorides and sulfates in the laboratory. It has been found that concentration by electrodialysis results in greater recoveries and requires less time of the investigator than the conventional absorption on Norite A or Alumina with its subsequent filtrations and elutions. Both procedures effect a certain degree of purification dependent on the type of impurities. Similar results are also achieved with Bacitracin. Only one type of electrodialysis used by the author will be discussed in this article.

EXPERIMENTAL

Equipment.—The requisite equipment may be of the simplest variety; two cylindrical platinum screen electrodes (50 mm. length x 12 mm. diameter), a glass stirrer, a source of direct current capable of delivering 300 milliamperes at 450 volts, suitable dialyzing tubing, and a means of reading milliamperes and volts.

Method.—The aqueous sample is placed in a convenient glass container; the two electrodes, 60 to 70 mm. apart, housed in cellophane bags are lowered into the solution along with a stirrer, and the current adjusted to the desired level. The cellophane bags were made from tubing that was 8.4 cm. in circumference. Each bag was tied with string to the upper shaft of the electrode and was punctured at a point well above the level of the liquid to permit the escape of evolved gases. The cell contents were maintained at 15 to 20° by means of an outer bath. By utilizing a hypodermic syringe and needle, samples may be removed from either the anolyte or catholyte without interrupting the current.

Streptomycin.—Mixtures of Streptomycin sulfate and chloride solutions containing approximately 1,000,000 units in 200 ml. have been electrodialyzed and a recovery of 85–90% achieved in three hours using a current of 240 milliamperes at 430 volts. The free base collected in the catholyte was neutralized with the desired mineral acid. The size of the cellophane bags was such that they conveniently

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held 20 to 30 ml. thus affording a seven- to tenfold concentration. When Streptomycin calcium chloride complex was so treated, it was noted that the catholyte took on an amber color during the electrodialysis and the resultant recovery was low (60 to 70%). Apparently the accumulation of calcium hydroxide at the cathode is sufficient to cause some alkaline degradation as evidenced by the appearance of 5% (based on original amount of Streptomycin) free maltol in the catholyte after the electrodialysis of the complex. Preliminary experimentation indicates that the accumulation of alkali and alkaline earth ions in the catholyte may be circumvented by means of a circulating mercury cathode or a cell with several compartments separated by membranes of different porosities. Such devices would be particularly valuable for the direct removal of Streptomycin or Bacitracin from broths. These techniques will be discussed in a later publication.

Bacitracin.—When the Bacitracin is subjected to electrodialysis using a current of 100 milliamperes at 400 volts it appears to migrate to the cathode at least as readily as Streptomycin. This ease of dialysis is not indicative of a high molecular weight polypeptide. By this technique it has been possible to effect a marked increase in the purity of Bacitracin that was available to this laboratory. A sample assaying 37.5 units/mg. was electrodialyzed for four hours during which time the catholyte was syphoned off every hour and replaced with distilled water. The fractions were neutralized with dilute hydrochloric acid and dried from the frozen state. Of the four fractions, the third exhibited the highest potency, 58.6 units/mg., an increase of 55% over the original. Repeating the above fractional electrodialysis with the third fraction as starting material over a three-hour period, furnished a fraction assaying 62.2 units/mg. It is apparent that a cell with several compartments with membranes of different dialysis coefficients, the least permeable surrounding the cathode, would be advantageous. Further, it should be mentioned that the recovery data are significant. When 500 mg. of Bacitracin (37.5 units/mg.) in 200 ml. distilled water are electrodialyzed under the above conditions, 90% of the Bacitracin is recovered in one hour and 95% in two hours.

A unique application of this technique was found in the purification of a sample of Streptomycin which was highly contaminated with pyrogens. Repeated filtrations through Seitz filters failed to remove the pyrogens. Finally, the sample was electrodialyzed. The cellophane bag used for collecting the catholyte was heated overnight at 102°; aseptic pyrogen-free water was used to fill the catholyte bag, and the syringe and glass container for storing the electrodialyzed Streptomycin were pyrogen-free. The resulting assays indicated that the pyrogenic content had been reduced to a negligible level.

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1949 Iodine Research Award Nominations Requested

Nominations are now being received by the AMERICAN PHARMACEUTICAL ASSOCIATION for the 1949 Iodine Educational Bureau Award recognizing outstanding research in the chemistry and pharmacy of iodine and its compounds as applied in pharmacy or medicine. Any member of the ASSOCIATION may propose a nominee by submitting eight copies of each of the publications to be considered in the competition, a biographical sketch of the nominee including date of birth, and a list of his publications. Eight copies of the nomination must be submitted to the Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION, Dr. Robert P. Fischelis, 2215 Constitution Ave., N. W., Washington 7, D. C. To be eligible for the 1949 Award, nominations must be received before January 1.

Establishment of the iodine award was first announced at the Milwaukee convention of the AMERICAN PHARMACEUTICAL ASSOCIATION which administers the competition.

A nominee must be a resident of the United States or Canada. He must have accomplished outstanding research in the chemistry or pharmacy of iodine and its compounds as applied in pharmacy or medicine.

During the period covered by the nomination the nominee shall have been actively engaged in, shall have completed, or shall have published a report upon the line of investigation for which the award is made. During a period of two years prior to the date of nomination, the nominee shall not have been engaged in research under the sponsorship of the Iodine Educational Bureau, Inc.

The award consists of $1000 and a diploma setting forth the reasons for selection of the recipient. It may be presented annually at the annual meeting of the AMERICAN PHARMACEUTICAL ASSOCIATION.

The recipient will deliver a paper or lecture upon the subject of his scientific work at the meeting at which the award is conferred. His paper, or address, will then be published in the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION. In addition to the sum of the award, the recipient will receive an allowance of not more than $250 to defray his expenses in attending the meeting.

The recipient will be selected by an award committee which is appointed by the chairman of the ASSOCIATION's Council and which functions under prescribed rules.

Should the award committee fail to find a suitable recipient in any year, two awards may be made during the next year if suitable recipients are selected.

A New Absorption Delaying Vehicle for Penicillin

By F. H. BUCKWALTER and H. L. DICKISON

THE NEED for a repository form of penicillin which could be injected less frequently than aqueous penicillin solutions and still maintain therapeutic blood levels was recognized early.

Although several methods have been proposed (1–10), the most successful, until recently, has been the familiar peanut oil–beeswax formulation introduced by Romansky and Rittman (1). This product utilizes the principle of a delaying vehicle with a soluble penicillin salt.

In our search for a satisfactory repository vehicle, we set up the following characteristics which this vehicle should possess:

1. It must be nontoxic, nonirritating, and nonallergenic.

2. It must not affect adversely the stability of penicillin.

3. It must possess a viscosity to permit withdrawal into a syringe and administration at room temperature.

4. It should retard the rate of release of penicillin from the site of injection.

5. It should be of such consistency as to prevent settling of suspended penicillin particles.

It was found that gels of aluminum stearates and vegetable oils would meet most of the above requirements. The viscosity of those gels will depend on which aluminum stearate (mono, di-, or tri-) is used, the amount of the aluminum stearate, the type of oil, and the conditions under

TABLE I.—BLOOD LEVEL STUDIES IN RABBITS USING SODIUM PENICILLIN G AND PROCAINE PENICILLIN G

Product	Rabbit	Blood Levels, Hr. 1	4	24	28	48	77	96	100	150	174	198	222	242
A	106	..	4.8	0.15	0.04	..	..	..	..	..	..	....	....	..
	107	..	4.4	0.30	0.07	..	..	..	..	..	..	....	....	...
	108	..	4.6	0.18	0.04	..	..	..	..	..	..	....	....	...
	110	..	3.4	0.11	NR	..	..	..	..	..	..	....	....	...
	130	..	5.0	0.10	NR	..	..	..	..	..	..	....	....	...
	131	..	0.8	0.55	0.14	..	..	..	..	..	..	....	....	...
	132	..	2.2	0.21	0.05	..	..	..	..	..	..	....	....	..
	133	..	3.3	0.08	NR	..	..	..	..	..	..	....	....	...
	134	..	2.4	0.04	NR	..	..	..	..	..	..	....	....	...
	135	..	3.8	0.59	0.04	..	..	..	..	..	..	....	....	...
B	166	..	..	0.08	0.04	NR	..	..	..	..	..	....	....	...
	168	..	..	0.56	0.08	NR	..	..	..	..	..	....	....	...
	51	..	4.4	0.15	0.10	NR	..	..	..	..	..	....	....	...
	172	..	2.45	0.22	0.12	0.05	..	..	..	..	..	....	....	...
	173	..	1.55	0.09	0.03	NR	..	..	..	..	..	....	....	...
	174	..	2.45	0.58	0.28	0.13	..	..	..	..	..	....	....	..
	175	..	1.85	0.43	0.06	NR	..	..	..	..	..	....	....	...
	176	..	3.05	0.67	0.07	0.07	..	..	..	..	..	....	....	..
C	372	7.30	2.75	0.03	NR	NR	..	..	..	..	..	....	....	...
	373	4.85	3.55	0.09	0.04	NR	..	..	..	..	..	....	....	...
	374	3.50	2.45	0.24	0.14	NR	..	..	..	..	..	....	....	...
	375	6.50	2.98	<0.01	0.02	NR	..	..	..	..	..	....	....	...
	376	9.85	4.95	NR	NR	0.02	..	..	..	..	..	....	....	...
	377	6.75	3.83	NR	NR	NR	..	..	..	..	..	....	....	...
	378	7.10	3.05	0.40	0.21	NR	..	..	..	..	..	....	....	...
	379	5.40	1.53	0.8	0.11	NR	..	..	..	..	..	....	....	...
	380	7.00	3.10	0.07	<0.01	NR	..	..	..	..	..	....	....	...
	381	6.85	2.65	NR	NR	NR	..	..	..	..	..	....	....	...
D	365	NR	0.85	0.43	0.17	0.15	0.09	..	0.06	..	..	....	....	...
	366	NR	1.05	0.25	0.20	0.18	0.08	..	0.07	..	..	....	....	...
	368	NR	1.1	0.39	0.18	0.17	0.07	..	0.05	..	..	....	....	...
	370	NR	0.78	0.54	0.22	0.09	0.03	..	NR	..	..	....	....	...
	371	NR	3.05	0.45	0.21	0.11	0.05	..	..	..	..	....	....	...
E	382	0.30	0.17	0.18	0.2	0.27	0.20	0.23	..	0.04	0.04	NR	NR	...
	383	0.60	0.22	0.31	0.27	0.42	0.18	0.24	..	0.10	0.09	<0.03	0.03	NR
	384	0.35	0.14	0.19	0.14	0.20	0.06	0.12	..	0.06	0.05	<0.03	<0.03	NR
	385	0.63	0.28	0.25	0.27	0.26	0.24	0.37	..	0.09	Died	....	....	...
	386	0.66	0.25	0.35	0.34	0.32	0.17	0.15	..	0.07	0.06	NR	NR	...
	387	0.27	0.09	0.19	0.18	0.17	0.20	0.20	..	0.11	0.06	0.05	NR	...
	388	0.74	0.18	0.29	0.21	0.23	0.13	0.24	..	0.06	NR	NR	NR	...
	389	0.41	0.15	0.19	0.25	0.20	0.23	0.29	..	0.10	0.06	NR	NR	...
	390	0.86	0.28	0.23	0.3	0.26	0.20	0.25	..	0.06	0.05	NR	NR	...
	391	0.49	0.26	0.3	0.21	0.33	0.25	0.19	..	NR	NR	NR	NR	...

NR—Indicates no zones of inhibition on plates.

	Particle Size
A—Crystalline sodium penicillin G in peanut oil containing beeswax (Romansky type)	67% over 50 microns
B—Crystalline sodium penicillin G in peanut oil gelled wtih 2% aluminum monostearate	70% over 50 microns
C—Procaine penicillin in peanut oil	93% over 50 microns
D—Procaine penicillin in peanut oil gelled with 2% aluminum monostearate	79% over 50 microns
E—Procaine penicillin in peanut oil gelled with 2% aluminum monostearate	98% less than 5 microns

which the gel is made. Since these gels possess a high degree of water repellency, it was reasoned they should be of particular value for parenteral use in delaying the release of suspended penicillin and hence prolonging blood levels. These gels possess thixotropic properties which effectively prevent settling of suspended penicillin particles. No discernible settling is evident after centrifuging five minutes at 1000 r. p. m. Extensive work with these gels indicated they had no detectable effect on the stability of the suspended penicillin salts. Their viscosities were such as to enable easy withdrawal into a syringe and administration at room temperature.

Peanut oil gels containing 2 per cent aluminum monostearate and various penicillin salts were tested in rabbits for blood levels. In each case. the products contained 300,000 units of the penicillin salt per cc. of gel. We have studied in these gels crystalline sodium penicillin G, crystalline potassium penicillin G, calcium penicillin, aluminum penicillin, zirconium penicillin, procaine penicillin G, and various amine penicillins. We have studied the effect of particle size on duration of blood level. The results with large and small procaine penicillin crystals are presented in Table I and Fig. 1. The complete particle size studies with various penicillin salts will be presented in a later communication.

Table I gives the results of blood level studies in rabbits using the sodium penicillin G and procaine penicillin G formulations mentioned above. For comparison, results with sodium penicillin G in peanut oil and beeswax (Romansky type formula), and procaine penicillin G in oil are included. Complete results with all the formulations mentioned above will be presented elsewhere. For these studies, the rabbits were given intramuscular injections of 50,000 units/Kg. into the thigh muscles. We have found that this dosage of 50,000 units per kilogram correlates rather closely with the results obtained by daily intramuscular injections in man of 300,000 units.

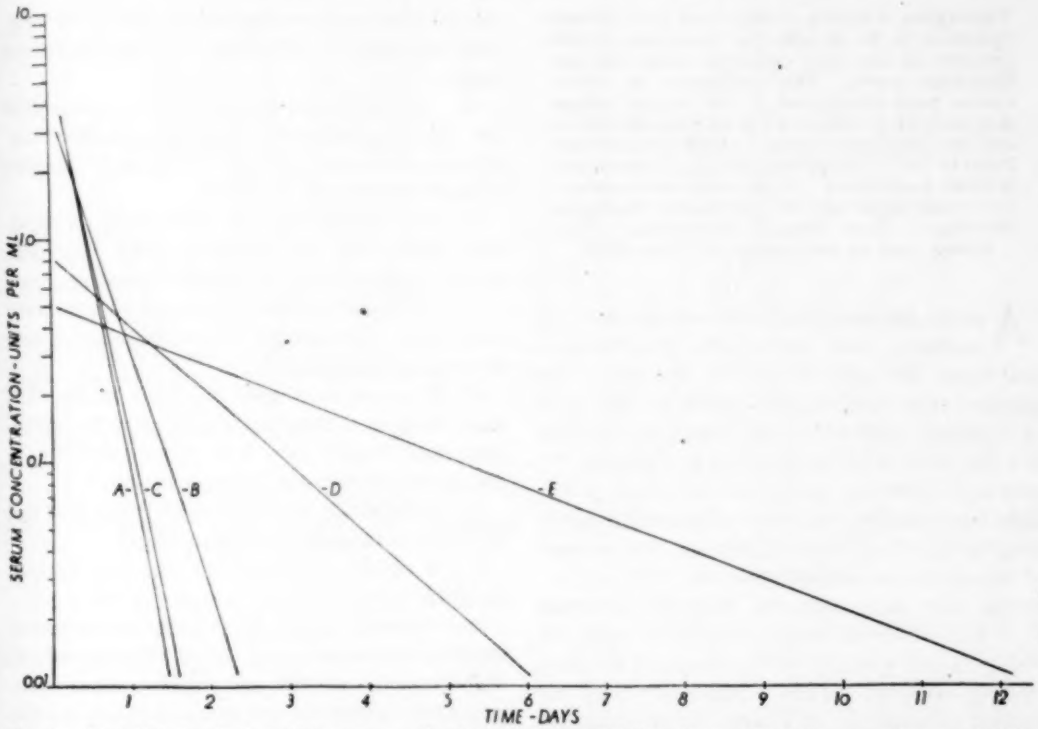

Fig. 1.—Penicillin levels in rabbit serum. Single injection—50,000 units per Kg. of suspensions containing 300,000 units per ml.

A, Sodium penicillin G in peanut oil containing beeswax.
B, Sodium penicillin G in aluminum stearate gel.
C, Procaine penicillin G in peanut oil.
D, Procaine penicillin G in aluminum stearate gel, large particles.
E, Procaine penicillin G in aluminum stearate gel, small particles.

The blood samples were assayed by a cup-plate method employing *Sarcina lutea* as the test organism. Pretreatment blood samples in all instances were negative. The effect of the vehicle is shown graphically in Fig. 1 where average blood levels are plotted against time in days. The submitted data are intended to show the super-

iority of peanut oil–aluminum stearate gels as repository forms for penicillin over peanut oil alone, or peanut oil–beeswax combinations.

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Sunscreen Compounds*

By W. D. KUMLER and T. C. DANIELS

The region in which a compound must absorb radiation to be an effective sunscreen is dependent on the solar radiation curve and the erythema curve. The ordinates of these curves were multiplied in the region where they overlap to obtain what we have chosen to call the "Sunburn Curve." This ranges from 2900 to 3260 Angstroms and has a maximum at 3080 Angstroms. Compounds with extinction coefficients two to eight times higher in this region than those of compounds commonly used as sunscreens are described.

AGENTS employed for protection against solar radiation have been divided into two general types, the physical and the chemical. The physical type contains such agents as zinc oxide or titanium oxide which are spread on the skin in a fine state of subdivision and by their opacity and high reflecting power prevent most of the light from reaching the skin. These compounds give the skin an artificial appearance and because of this are not considered desirable. We are concerned here only with the chemical sunscreen type which may be colorless and transparent to visible rays of sunlight but is opaque to the rays causing sunburn. These compounds may be applied as solutions, as lotions, or as ointments to leave a film that is not noticeable but which is effective in preventing sunburn.

The ideal sunscreen compound has to meet a wide variety of specifications.

1. It must absorb or filter out the rays causing sunburn which are those in the region from 2900 to 3300 Angstroms.

2. It should be stable in the presence of light, air, and moisture, or if it is decomposed under these conditions, the decomposition products should have comparable absorption to the original compound in the 2900- to 3300-Angstrom region.

3. It should have very slight or no absorption for the long ultraviolet rays beyond 3400 Angstroms which are thought to produce tanning without appreciable erythema.

4. The compound and decomposition products which may be produced under conditions of use should be nontoxic and nonirritating.

5. It should be nearly neutral so untoward effects are not produced by the presence of acid or base on the skin.

6. It should have good solubility in the ointment base or vehicle in which it is to be formulated and should have low water solubility to prevent rapid removal by perspiration.

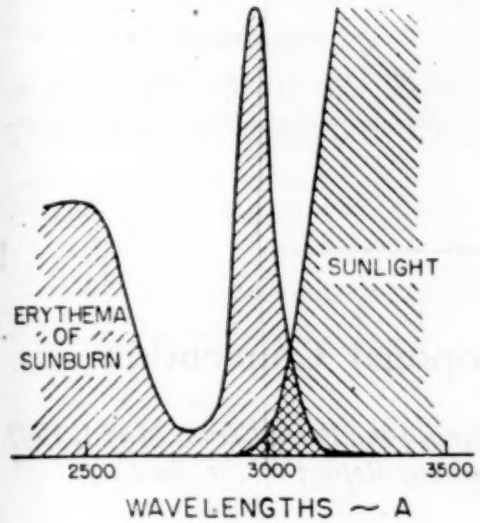
7. It should be relatively nonvolatile so it will not evaporate under conditions of use.

8. It should not be rapidly absorbed through the skin.

Two factors determine where a compound should absorb light to be an effective sunscreen. The first is the relative amount of erythema produced by various wave lengths of light, and the second is the energy distribution in the sunlight reaching the earth. Figure 1 shows the erythema-wave length curve (1) and the energy distribution of sunlight–wave length curve (2). It is apparent that man needs little or no protection below 2900 Angstroms. Although wave lengths around 2500 Angstroms are effective in producing erythema there are no rays shorter than 2900 Angstroms reaching the earth. The shorter wave lengths are screened out by the atmosphere. On the other hand, although the energy reaching the earth at wave lengths longer than 3300 is very high, one need not be protected from such rays

* Received August 11, 1948, from the College of Pharmacy, University of California, San Francisco, Calif.
Presented to the Scientific Section, A. PH. A., San Francisco meeting, August, 1948.

because they do not produce erythema in the normal person. In cases of people suffering from *Xeroderma pigmentosum*, *Hematoporphyria*, or *Hydroa aestivale* it is desirable to prepare special screens that absorb light of longer wave lengths as well as those in the erythema range. Normally such preparations will contain more than one absorbing agent. On the other hand, a screen to protect from the rays of a mercury vapor sterilization lamp, which yields over 80 per cent of its energy at 2537 Angstroms, requires a compound with high absorption at these short wave lengths. At present we will not consider these special cases but confine the discussion to sunscreens for the normal person.



From Coblentz and Stair, J. Research National Bureau of Standards

Fig. 1.—Erythema–wave length and sunlight energy–wave length curves.

Since the amount of sunburn produced by a given wave length of light is as a first approximation the product of the amount of light of that wave length reaching the skin times the relative effectiveness of that wave length in producing erythema, we have multiplied the ordinates of the erythema curve by those of the sunlight energy distribution curve and thus obtained the "Sunburn Curve" which is represented by the dotted line in Fig. 2. This curve shows graphically the wave lengths that should be screened out to prevent sunburn, and the height of the curve at any point gives an approximate indication of the relative importance of the various wave lengths in producing erythema.

The peak of this curve is at 3080 Angstroms. The higher the extinction coefficient of a compound at this wave length the greater will be the effectiveness of the compound as a sunburn preventive. In this respect the mono- and di-alkyl-para-aminobenzoate esters have very favorable extinction coefficients compared with compounds commonly used as sunscreens. Figure 2 shows the propitious relation of the extinction coefficient–wave length curves of these compounds to the "Sunburn Curve." These compounds have five to eight times the screening power of the salicylates and anthranilates, three times that of the umbelliferones and about twice that of the unsubstituted para-aminobenzoates. Ethyl-*p*-ethylaminobenzoate, which is an example of a mono-substituted compound, has an extinction

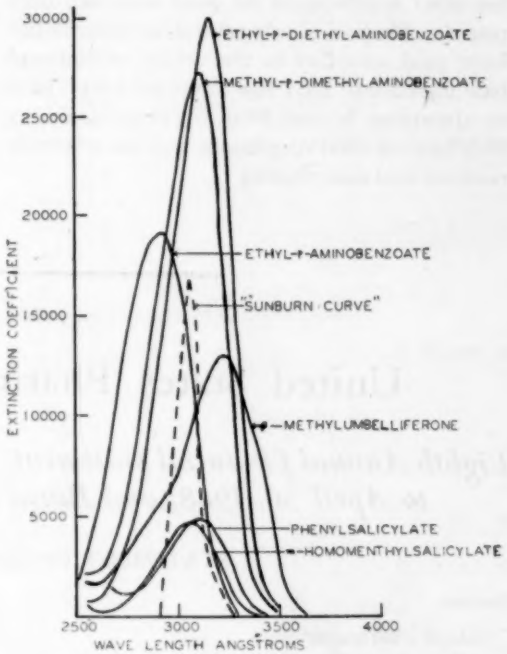


Figure 2.

coefficient of 23,200 at 3050 Angstroms which is very close to the maximum of the "Sunburn Curve." This compound does not have quite as high an extinction in this region, however, as some of the dialkylaminobenzoates. The latter compounds meet favorably the other specifications for an ideal sunscreen compound.

The dialkylaminobenzoate esters have good stability in the presence of light and air. This property is not shown by the salicylates or umbelliferones which have free phenolic hydroxy-groups, or by the anthranilates and *p*-aminobenzoates which have free aromatic amino groups. It is reported in the literature (3) that both *p* aminobenzoates and anthranilates discolor in sunlight and that a solution of menthyl salicylate retains only 50 per cent of its screening ability

after an exposure of four hours to the sun's rays. A solution of methyl-para-dimethylaminobenzoate contained in a Vycor tube remains colorless after being exposed four and a half hours either to sunlight or to the light of a mercury vapor lamp. The original optical density of the solution was 1.70 and after an exposure of four and a half hours one-half inch from a 100-watt mercury vapor lamp the density was unchanged. After exposure to direct sunlight from 11:40 A.M. to 4:20 P.M. the density was 1.65, only three per cent less than the original density, and this difference is within the experimental error of the measurements.

The para-dialkylaminobenzoate esters meet the other requirements for good sunscreen compounds. They are nearly neutral, are nonvolatile, have good solubility in the vehicle or ointment base ingredients, have low water solubility, have no absorption beyond 3500 Angstroms and very little beyond 3400 Angstroms, and are relatively nontoxic and nonirritating.

CONCLUSIONS

A "Sunburn Curve" has been calculated by multiplying the ordinates of the erythema curve by the ordinates of the sunlight energy distribution curve. The height of this curve gives an indication of the relative importance of various wave lengths in causing sunburn. The properties of an ideal sunscreen compound are described. The ultraviolet absorption spectrum of some mono- and dialkyl-para-aminobenzoic acid esters has been measured. These compounds are more effective sunscreens than the compounds commonly employed for this purpose. They are also quite stable in the presence of light and otherwise closely approach the requirements for an ideal sunscreen.

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United States Pharmacopœial Convention

Eighth Annual Financial Statement, Covering the Period from May 1, 1947 to April 30, 1948, and Based Upon the Report of the Auditor

STATEMENT OF INCOME AND EXPENSE

Income			
Sale of Pharmacopœias:			
Collections		$336,724.71	
Add: Increase in Accounts Receivable			
April 30, 1948	$20,609.74		
April 30, 1947	890.75	19,718.99	$356,443.70
Sale of Reference Standards, A. M. A. Articles, etc.:			
Collections		$ 13,711.45	
Add: Increase in Accounts Receivable			
April 30, 1948	$ 1,787.20		
April 30, 1947	803.00	984.20	
		$ 14,695.65	
Add: Increase in Collections Pending Remittance to the Treasurer			
April 30, 1948	$ 696.75		
April 30, 1947	487.75	209.00	14,904.65
Interest on Investments:			
Collections	$ 2,600.00		
Accrued Interest	1,242.31	$ 3,842.31	
Less: Amortization of Bond Premiums		88.21	3,754.10

Miscellaneous Income:				
Collections				2,473.80
Total Income				$377,576.25
Less—Expense				
Publication and Sales Expense		$90,044.64		
Less: Increase in Inventory				
April 30, 1948	$14,155.07			
April 30, 1947	6,475.99	7,679.08	$ 82,365.56	
Administration			5,029.28	
Revision		$68,119.21		
Less: Increase in Reference Standard Inventory				
April 30, 1948	$10,139.13			
April 30, 1947	6,033.75	4,105.38	64,013.83	
Research			11,358.62	
Convention			31.49	
Stationery and Supplies—Inventory Adjustment (Minus)			252.46	
Provision for Depreciation:				
Building		$ 346.71		
Furnishings and Equipment		359.45	706.16	
Loss on Disposal of Furnishings and Equipment			44.38	163,296.86
Excess of Income Over Expense				
For the year ended April 30, 1948				$214,279.39

STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS

Cash on Deposit, April 30, 1947			$ 47,646.56
Add—Receipts			
Sale of Pharmacopœias		$336,724.71	
Sale of Reference Standards, A. M. A. Articles, etc.		13,711.45	
Interest on Investments		2,600.00	
Use of Text by Others		2,140.00	
Miscellaneous (Including Rental of Office Space by Remington's Practice of Pharmacy—$300.00)		342.42	355,518.58
			$403,165.14

Less—Disbursements

	Publications and Sales	Administration	Revision	Research	Convention	Total
Printing and Binding	$90,044.64	.....	.....	.....	.....	$ 90,044.64
Salaries	.....	$1,500.00	$13,500.00	$ 6,908.31	.....	21,908.31
Honoraria	.....	200.00	21,700.00	.....	.....	21,900.00
Clerical	.....	1,248.00	8,518.20	151.65	$ 3.06	9,920.91
Meetings	.....	814.96	9,178.83	3,346.93	.....	13,340.72
Supplies	.....	65.50	1,372.58	886.61	.....	2,324.69
Postage and Telegrams	.....	196.73	1,328.15	32.12	28.43	1,585.43
Headquarters	.....	.....	1,447.18	.....	.....	1,447.18
General	.....	1,004.09	11,074.27	33.00	.....	12,111.36
	$90,044.64	$5,029.28	$68,119.21	$11,358.62	$31.49	$174,583.24

Add:		
Purchase of Furnishings and Equipment	$ 942.20	
Investments	150,652.69	326,178.13
Cash on Deposit, April 30, 1948		$ 76,987.01

A MORE DETAILED AND SUPPLEMENTARY STATEMENT OF THE REVISION AND RESEARCH EXPENSES

REVISION—MEETINGS

Conferences—Including Transportation, Hotel, Meals, etc., for Conferences Called or Made by the Chairman	$ 929.16
Meeting of the Revision Committee at Mount Pocono, the Scope Committee at New York City, and the Executive Committee at Philadelphia	8,249.67
	$ 9,178.83

REVISION—SUPPLIES

Stationery	$ 227.06
Mimeograph	606.05
Reproduction of Scope Voting Sheets for Bulletins	265.00
Miscellaneous	274.47
	$ 1,372.58

REVISION—HEADQUARTERS

Janitor (Part Time)	$ 827.35
Utilities, Supplies, Maintenance, Insurance	619.83
	$ 1,447.18

REVISION—GENERAL

Reference Standards	$ 7,402.67
Exhibits, etc.	2,381.94
A. PH. A. Abstracts	1,000.00
Miscellaneous	289.66
	$11,074.27

REVISION—HONORARIA

Name	Amount	Name	Amount
Walter A. Bastedo	$ 1,600.00	Loyd E. Harris	100.00
Cary Eggleston	1,600.00	Thomas J. Hill	100.00
E. E. Nelson	1,000.00	M. L. Jacobs	100.00
George W. McCoy	500.00	C. O. Lee	200.00
Heber W. Youngken	1,000.00	A. B. Lemon	100.00
Joseph Rosin	2,500.00	E. V. Lynn	100.00
John C. Krantz, Jr.	1,600.00	Perrin H. Long	100.00
George D. Beal	2,000.00	E. K. Marshall, Jr.	100.00
Glenn L. Jenkins	500.00	W. L. Mendenhall	100.00
B. V. Christensen	500.00	Lloyd C. Miller	100.00
Francis E. Bibbins	500.00	Hugh C. Muldoon	100.00
H. A. Langenhan	500.00	E. L. Newcomb	200.00
Leonard A. Seltzer	300.00	C. Leonard O'Connell	100.00
Louis C. Zopf	300.00	Justin L. Powers	200.00
Frank O. Taylor	1,000.00	J. Allen Reese	100.00
A. G. DuMez	1,000.00	Leon W. Richards	100.00
Charles L. Brown	200.00	William T. Salter	100.00
Joseph B. Burt	100.00	Hugo H. Schaefer	300.00
W. B. Castle	500.00	George C. Schicks	100.00
C. W. Chapman	100.00	Carl L. A. Schmidt	100.00
Arthur C. DeGraff	100.00	Isaac Starr	100.00
M. S. Dooley	200.00	George W. Thorn	100.00
Carl A. Dragstedt	100.00	Elmer H. Wirth	300.00
Robert P. Fischelis	100.00	Louis Gershenfeld	100.00
P. A. Foote	100.00	C. T. Van Meter	100.00
Harry Gold	300.00	J. A. Bianculli	100.00
Forest J. Goodrich	100.00		
Harvey B. Haag	100.00		$21,700.00

RESEARCH

Subcommittees	Meetings, Supplies, Postage, Clerical, Travel and General	Technical Assistance
No. 1—Scope	$ 89.76 (Not including meeting of entire Committee)	
No. 3—Bio Assays	378.33	
No. 5—Botany and Pharmacognosy	39.89	$ 50.00
No. 7—Inorganic Chemistry	157.42	2,791.65
No. 8—Organic Chemistry	1,000.00	3,466.66
No. 13—Ointments	1,293.92	600.00
Amino Acids Committee	354.49	
Anti-anemia Board	463.22	
Endocrine Advisory Board	552.08	
Sterile Products Board	18.45	
Vitamin Board	102.75	
	$4,450.31	$6,908.31

Note on the Microcrystallographic Properties of Rutin, Quercitrin and Quercetin*

By GEORGE L. KEENAN†, ‡

RUTIN, $C_{27}H_{30}O_{16}.2H_2O$, a glycosidic principle, is found in rue (*Ruta graveolens*,L.) and many other plants, and is of interest in that it is supposed to have an important effect on resolving arteriosclerosis and other functions of assimilation. The lack of microscopic data on the substance initiated the present study for purposes of record, also including a similar investigation in respect to quercitrin and the flavone, quercetin, the latter being one of the products of hydrolyzing both rutin and quercitrin.

EXPERIMENTAL

Rutin is lemon-yellow and the microscopic examination in ordinary light shows that it consists of very minute rods, almost too small for satisfactory inspection. Therefore, it was found more practicable to crystallize the material from alcohol, thus obtaining larger rods. Rutin crystallizes with 1 mol. of solvent of crystallization from alcohol. With crossed nicols, the extinction is parallel and the sign of elongation is negative. The rods were not large enough to reveal interference figures with the conoscopic examination. The significant refractive indices are: $\alpha = 1.508$ (commonly shown lengthwise on rods), $\beta = 1.734$ (commonly shown crosswise and apparently the β-value although an interference figure could not be obtained) $\gamma = >1.734$. All ± 0.002.

Quercitrin, $C_{21}H_{20}O_{11}.2H_2O$ is the glycoside found in the inner bark of the black oak (*Quercus velutina*, Lam.) and other plants. It is orange-yellow and on hydrolyzing, quercetin, in addition to rhamnose, is formed. In ordinary light, the microscopic examination shows that the material is characterized by thin plates, many of which are elongated. These thin plates give a glistening effect to the powder. With crossed nicols, the extinction is parallel, the plates invariably extinguishing sharply, therefore precluding any interference figures with the conoscopic examination. The significant refractive indices are: $\omega = 1.508$ (frequently shown on the elongated plates when their long axis is parallel to the vibration plane of the lower nicol), $\epsilon = >1.734$. Both ± 0.002.

Quercetin, $C_{15}H_{10}O_7.2H_2O$, the aglucone of rutin, is yellow and consists of clusters of spindle-shaped crystals. These clusters are extremely small and best observed in ordinary light at high magnification (4 mm. objective and 10× ocular found satisfactory). Broken fragments of the spindle shaped crystals are frequently observed in the preparation. With crossed nicols the spindle-shaped crystals extinguish sharply when their long axis is parallel to the plane of vibration of the lower nicol (lengthwise). The sign of elongation is negative. The crystals are too small to show interference figures with the conoscopic examination. The significant refractive indices are: $\alpha = 1.555$ (frequently shown when the spindle-shaped crystal or fragment is oriented with its long axis parallel to the vibration plane of the lower nicol), $\beta = 1.734$ (not at all common and apparently shown when the spindles are tipped on edge), $\gamma = >1.734$. All ± 0.002.

* Received March 22, 1948.

† Present address: 165 Westwood Drive, Strongsville, Ohio.

‡ The writer gratefully acknowledges the cooperation of Dr. James F. Couch, Eastern Regional Research Laboratory, U. S. Department of Agriculture, Philadelphia, Pa., in furnishing the materials for this study; also that of Baldwin-Wallace College, Berea, Ohio.

Book Reviews

Law of Drugs and Druggists. 3rd ed. By WILLIAM R. ARTHUR. West Publishing Company, St. Paul, Minn., 1947. xxxvii + 702 pp. 15 x 23 cm. Price $5.00.

Books on pharmaceutical law generally fall into two classes: those written by lawyers who have developed a special interest in pharmaceutical law, and those written by pharmacists who have developed an interest in jurisprudence. The former tend to be heavy with legal terminology and lacking in pharmaceutical applications, while the latter, avoiding these two disadvantages, tend to contribute insufficient legal material to qualify as authoritative works on pharmaceutical law.

"Law of Drugs and Druggists," while definitely belonging to the first of these two classes, is the best approach to a pharmaceutical explanation of the laws pertaining to pharmacy which has appeared to date. The legal language is there, but the book includes a good glossary of the legal terms. The large number of cases used to illustrate the legal topics are all pharmaceutically pertinent or are determining cases related to the origin of the rules of law. There still remains some slight lack of pharmaceutical emphasis, however. For example, while the book is intended for "retail, wholesale and manufacturing druggists" as well as students of pharmacy, the treatment of alcohol is restricted to the legal problems arising from its use in filling prescriptions, to the neglect of considerations arising from its use in manufacturing (at the retail, hospital, or industrial level), or of considerations of the Federal alcohol tax and its draw-back provisions. This is perhaps understandable, however, as too great a degree of completeness would make the book unwieldy to use, in view of the large amount of legal material to be covered.

Pharmaceutical law is, to some extent, criminal law. It includes some principles of tort law. Pharmacists are controlled in the practice of their profession by 49 constitutions, a Federal and a state system of laws, as well as a host of additional local regulations. There is also, in most states, the common law, "a system of principles and rules enunciated by the courts in conformity with immemorial custom and usage." There has also developed a large body of administrative law based upon the decisions of quasi-judicial bodies such as the state boards, the Federal Food Drug and Cosmetic Administration, the Federal Trade Commission and others. There are thousands of court decisions affecting the drug business.

"Law of Drugs and Druggists" is perhaps the only book which attempts to deal with the entire subject of pharmaceutical law, and it approaches the task in a very effective manner. The various legal topics, as they are taken up, are illustrated by digests of actual cases, rather than by discussion. This device has the disadvantage of making the book appear to a great extent to consist of excerpts or quotations from statutes and court decisions. On the other hand, in addition to being concise, it has the great advantage of giving basic material of primary authority—a statement of what the law is rather than what some commentator thinks about it. However, it causes the book to take on some of the characteristics of a reference work. While this is excellent for the pharmacist and teacher, it does increase the difficulty of the student attempting to use the work as a textbook.

The third edition is greatly enlarged and expanded over the previous one. Additional cases have been added and it has been brought up to date on laws governing the vitamins, on the administrative provisions under the Food, Drug, and Cosmetic Act, the Wheeler-Lea Act and others. Over 225 digests of cases and nearly 500 portions of other cases are used to clarify specific points under definite topics. In addition there are over 425 reading references for further material on specific topics. Questions on the cases used are also included for the convenience of teacher and student. Further aids for the student, in addition to the glossary of legal terms, include a chapter on special rules of law as they apply to drug cases, a chapter on special problems of the druggist, an explanation of the different kinds of courts and their jurisdictions, and an explanation of the legal reporter systems and their reports.

A wealth of basic material is presented in this book.—STEPHEN WILSON.

The Chemical Senses. By R. W. MONCRIEFF. John Wiley and Sons, New York, 1946. vii + 424 pp. 14 x 21.5 cm. Price $4.50.

The special senses are physiologically characterized according to their typical responses to mechanical, physical, and chemical stimuli. Many tests are available which describe these senses from the physiological approach. This monograph by Moncrieff is a chemical approach to the senses of smell and taste, which are responsive to external chemical stimuli.

The author introduces the reader to this subject through an introductory chapter on the role of the chemical senses in life in general. This is followed by the anatomic functional physiology of the sense organs proceeding from the general to the specific and including the chemical sensibility of lower animals.

Various schemes for classifying odors are outlined in a short chapter. It is noteworthy that at least one of these schemes is capable of semiquantitative expression.

Two chapters on chemical constitution in its relation to odor and taste will be of special interest to the organic chemist.

Physical properties of odorous material, theories of odor, a chapter on perfumes and essences and one on flavor and food are also covered.

The book is well documented, the references appearing throughout the text rather than segregated. It is well indexed also. While the book often leaves something to be desired in the practical translation of scientific data into technological use, it is a helpful guide to a fascinating field.